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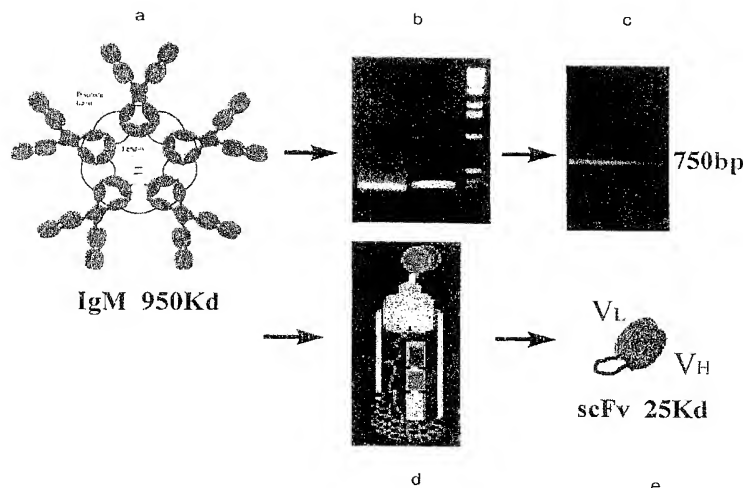
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[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS FOR THE TREATMENT AND/OR DIAGNOSIS OF NEUROLOGICAL DISEASES AND DISORDERS



(57) Abstract: A method of immunizing against plaque forming diseases using display technology is provided. The method utilize novel agents, or pharmaceutical compositions for vaccination against plaque forming diseases which rely upon presentation of an antigen or epitope on a display vehicle. The method further includes agents, or pharmaceutical compositions for vaccination against plaque forming diseases, which rely upon presentation of an antibody, or an active portion thereof, on a display vehicle. Whether antigens or antibodies are employed, disaggregation of plaques results from the immunization. The methods of the present invention also generally relates to treating and/or diagnosing neurological diseases and disorders of the central nervous, regardless of whether the disease or disorder is plaque-forming or non-plaque forming.



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METHODS AND COMPOSITIONS FOR THE TREATMENT AND/OR DIAGNOSIS OF
NEUROLOGICAL DISEASES AND DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This is related to U.S. Patent Application 09/629,971, filed July 31, 2000, which is a continuation-in-part of U.S. Patent Application No. 09/473,653, filed December 29, 1999, which claims priority under 35 U.S.C. §119(e) from U.S. Provisional Patent Application No. 60/152,417, filed September 3, 1999. The entire contents of all three applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The present invention relates to agents and compositions and to methods for treating a neurological disease or disorder of the central nervous system (CNS), such as a plaque-forming disease. More particularly, the methods according to the present invention involve the use of (i) plaque derived antigens cloned and displayed on the surface of a display vehicle for *in vivo* elicitation of antibodies capable of preventing plaque formation and of disaggregating existing plaques; and (ii) antibodies raised against plaque derived antigens, at least an immunologic portion of which is

cloned and displayed on a display vehicle, which immunologic portion is capable of preventing plaque formation and of disaggregating existing plaques. The present invention also relates to a method of targeting a display vehicle to the brain of an animal, including man, and to a method for detecting the presence of plaque-forming prions. The present invention further relates to a method of diagnosing the presence or extent of a neurological disease or disorder of the central nervous system by *in vivo* imaging.

Description of the Related Art

[0003] Plaques forming diseases are characterized by the presence of amyloid plaques deposits in the brain as well as neuronal degeneration. Amyloid deposits are formed by peptide aggregated to an insoluble mass. The nature of the peptide varies in different diseases but in most cases, the aggregate has a beta-pleated sheet structure and stains with Congo Red dye. In addition to Alzheimer's disease (AD), early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease, other diseases characterized by amyloid deposits are, for example, SAA amyloidosis, hereditary Icelandic syndrome, multiple myeloma, and prion diseases. The most common prion diseases in animals are scrapie of sheep and goats and bovine spongiform encephalopathy (BSE) of cattle (Wilesmith and Wells, 1991).

Four prion diseases have been identified in humans: (i) kuru, (ii) Creutzfeldt-Jakob Disease (CJD), (iii) Gerstmann-Streussler-Sheinker Disease (GSS), and (iv) fatal familial insomnia (FFI) (Gajdusek, 1977 and Medori et al., 1992).

Etiology of prion diseases:

[0004] Prion diseases involve conversion of the normal cellular prion protein (PrP^{C}) into the corresponding scrapie isoform (PrP^{Sc}). Spectroscopic measurements demonstrate that the conversion of PrP^{C} into the scrapie isoform (PrP^{Sc}) involves a major conformational transition, implying that prion diseases, like other amyloidogenic diseases, are disorders of protein conformation. The transition from PrP^{C} to PrP^{Sc} is accompanied by a decrease in α -helical secondary structure (from 42% to 30%) and a remarkable increase in β -sheet content (from 3% to 43%) (Caughey et. al. 1991, Pan et. al. 1993). This rearrangement is associated with abnormal physiochemical properties, including insolubility in non-denaturing detergents and partial resistance to proteolysis. Previous studies have shown that a synthetic peptide homologous with residues 106-126 of human PrP ($\text{PrP}_{106-126}$) exhibits some of the pathogenic and physicochemical properties of PrP^{Sc} (Selvaggini et. al. 1993, Tagliavini et. al. 1993, Forloni, et. al. 1993). The peptide shows a remarkable conformational polymorphism, acquiring different secondary

structures in various environments (De Gioia et al. 1994). It tends to adopt a β -sheet conformation in buffered solutions, and aggregates into amyloid fibrils that are partly resistant to digestion with protease. Recently, the x-ray crystallographic studies of a complex of antibody 3F4 and its peptide epitope (PrP 104-113) provided a structural view of this flexible region that is thought to be a component of the conformational rearrangement essential to the development of prion disease (Kanyo et al. 1999). The identification of classes of sequences that participate in folding-unfolding and/or solubilization-aggregation processes may open new direction for the treatment of plaque forming disease, based on the prevention of aggregation and/or the induction of dissaggregation (Silen and Agard, 1989, Frenkel et al. 1998, Horiuchi and Caughey, 1999).

Alzheimer's disease - clinical overview:

[0005] Alzheimer's disease (AD) is a progressive disease resulting in senile dementia. Broadly speaking, the disease falls into two categories: late onset, which occurs in old age (typically above 65 years) and early onset, which develops well before the senile period, e.g., between 35 and 60 years. In both types of the disease, the pathology is similar, but the abnormalities tend to be more severe and widespread in

cases beginning at an earlier age. The disease is characterized by two types of lesions in the brain, senile plaques and neurofibrillary tangles. Senile plaques are areas of disorganized neurofibrils up to 150 nm across with extracellular amyloid deposits at the center, visible by microscopic analysis of sections of brain tissue. Neurofibrillary tangles are intracellular deposits of tau protein consisting of two filaments twisted about each other in pairs.

Senile plaques and other amyloid plaques:

[0006] The principal constituent of the senile plaques is a peptide termed A β or beta-amyloid peptide (β AP). The amyloid beta peptide is an internal fragment of 39-43 amino acids of a precursor protein termed amyloid precursor protein (APP). Several mutations within the APP protein have been correlated with the presence of Alzheimer's disease (See, e.g., Goate et al., 1991, valine⁷¹⁷ to isoleucine; Harlan et al. 1991, valine⁷¹⁷ to glycine; Murrell et al., 1991, valine⁷¹⁷ to phenylalanine; Mullan et al., 1992, a double mutation, changing lysine⁵⁹⁵ -methionine⁵⁹⁶ to asparagine⁵⁹⁵ -leucine⁵⁹⁶).

[0007] Such mutations are thought to cause Alzheimer's disease by increased or altered processing of APP to beta-

amyloid, particularly processing of APP to increased amounts of the long form of beta-amyloid (i.e., A β 1-42 and A β 1-43). Mutations in other genes, such as the presenilin genes, PS1 and PS2, are thought indirectly to affect processing of APP to generate increased amounts of long form beta-amyloid (see Hardy, 1997). These observations indicate that beta-amyloid, and particularly its long form, is a causative element in Alzheimer's disease.

[0008] Other peptides or proteins with evidence of self aggregation are also known, such as, but not limited to, amylin (Young et al., 1994); bombesin, caerulein, cholecystokinin octapeptide, eledoisin, gastrin-related pentapeptide, gastrin tetrapeptide, somatostatin (reduced), substance P; and peptide, luteinizing hormone releasing hormone, somatostatin N-Tyr (Banks and Kastin, 1992).

[0009] Binding of high affinity monoclonal antibodies (mAbs) to such regions may alter the molecular dynamics of the whole protein chain or assembly. By appropriate selection, mAbs have been found to recognize incompletely folded epitopes and to induce native conformation in partially or wrongly folded protein (Frauenfelder et al. 1979; Blond and Goldberg 1987; Karplus and Petsko 1990; Carlson and Yarmush 1992; Solomon and Schwartz 1995).

Treatment:

[0010] U.S. Pat. No. 5,688,561 to Solomon teaches methods of identifying monoclonal antibodies effective in disaggregating protein aggregates and preventing aggregation of such proteins. Specifically, U.S. Pat. No. 5,688,561 demonstrates anti-beta-amyloid monoclonal antibodies effective in disaggregating beta-amyloid plaques and preventing beta-amyloid plaque formation *in vitro*. U.S. Pat. No. 5,688,561 stipulates the *in vivo* use of such antibodies to prevent plaque formation by aggregation of beta-amyloid or to disaggregate beta-amyloid plaques which have already formed. These teachings do not, however, identify an epitope to be employed to generate such antibodies. In addition, these teachings do not provide means with which to enable the penetration of such antibodies into the brain through the blood brain barrier (BBB). Furthermore, this patent fails to teach the use of phage display technology as a delivery method for antigens or antibodies. Yet furthermore, no experimental results demonstrating the *in vivo* effectiveness of such antibodies are demonstrated by U.S. Pat. No. 5,688,561.

[0011] EP 526511 by McMichael teaches administration of homeopathic dosages (less than or equal to 10^{-2} mg/day) of beta-amyloid to patients with pre-established AD. In a typical human with about 5 liters of plasma, even the upper

limit of this dosage would be expected to generate a concentration of no more than 2 pg/ml. The normal concentration of beta-amyloid in human plasma is typically in the range of 50-200 pg/ml (Seubert et al., 1992). Because this proposed dosage would barely alter the level of endogenous circulating beta-amyloid and because EP 526511 does not recommend the use of an adjuvant, it seems implausible that any therapeutic benefit would result therefrom.

[0012] PCT/US98/25386 by Schenk and a Nature paper by Schenk et al., 1999) teach administration of beta-amyloid immunogens to a patient in order to generate antibodies to prevent formation of plaques or dissolve existing plaques. According to Schenk, 50 to 100 mg of antigen are required, 1 to 10 mg if an adjuvant is employed. These teachings also stipulate that a similar effect may be achieved by direct administration of antibodies against beta-amyloid, in both cases disregarding the blood brain barrier which, under normal circumstances, prevents the penetration of antibodies into the brain.

[0013] It is also important to note that these teachings are typically restricted to the use of "...any of the naturally occurring forms of beta-amyloid peptide, and particularly the human forms (i.e., A β 39, A β 40, A β 41, A β 42 or A β 43)" or "...longer polypeptides that include, for example, a beta-

amyloid peptide, active fragment or analog together with other amino acids", or "multimers of monomeric immunogenic agents".

[0014] These teachings ignore, however, earlier data teaching that the first 28 amino acids of beta-amyloid are sufficient to elicit antibodies which both disaggregate and inhibit aggregation of beta-amyloid plaques *in vitro* (Hanan and Solomon, 1996; Solomon et al., 1996; and Solomon et al., 1997).

[0015] Schenk and Schenk et al. both fail to teach the use of the N-terminal epitope of beta-amyloid plaques which is known to be a sequential epitope composed of only four amino acid residues (EFRH, SEQ ID NO:1) located at positions 3-6 of the beta-amyloid peptide (Frenkel, 1998). Antibodies against this epitope have subsequently been shown to disaggregate beta-amyloid fibrils, restore beta-amyloid plaques solubilization and prevent neurotoxic effects on PC 12 cells (Solomon, B. et al., 1997 and Solomon, B., et al., 1996).

[0016] This epitope has been independently confirmed as the epitope bound by anti-aggregating antibodies using random combinatorial hexapeptide phage display (Frenkel and Solomon, J. of Neuroimmunol. 88:85-90, 1998).

[0017] The EFRH (SEQ ID NO:1) epitope is available for antibody binding when beta-amyloid peptide is either in solution or in aggregates. Blocking of this epitope by a

monoclonal antibody prevents self-aggregation and enables resolubilization of already formed aggregates.

[0018] These findings suggest that the teachings of Schenk and colleagues are inefficient at best. Since, as has already been mentioned hereinabove, the normal concentration of beta-amyloid in human serum is 50-200 pg/ml, immunization with that peptide could be expected to produce either low antibody titers or high toxicity if strong adjuvants are used and as such it is not applicable for therapy. Indeed, in order to achieve significant serum titers of antibody against beta-amyloid a series of 11 monthly injections was required (Schenk *et al.*, Nature, 400:173-177, 1999). The degree to which these serum titers will persist over time is not yet known, and this point is especially crucial with respect to early onset Alzheimer's disease.

[0019] Schenk and colleagues further teach that an immunogenic peptide such as beta-amyloid may be displayed upon the surface of a virus or bacteria. However, they fail to teach use of an antigen so displayed to effect immunization. No mention is made of defining an epitope in this context and no experimental data is provided either. In addition, delivery of antibody displayed on a display vehicle is not taught by Schenk or Schenk *et al.* altogether.

[0020] Collectively, the prior art fails to teach means with which an effective titer of anti-aggregation antibodies can be generated *in vivo* in a short time and/or be introduced into the brains of patients suffering a plaque-forming diseases. In addition, the persistence of titers generated via prior art teachings has not been established.

[0021] There is thus a widely recognized need for, and it would be highly advantageous to have, effective means of disaggregating amyloid plaques *in vivo* which would have lasting effect, high efficiency, rapid onset, no adverse effect on the treated subject and which is readily amenable to large scale production.

Blood Brain Barrier:

[0022] The blood-brain barrier (BBB) (Johansson, 1992; Ermisch, 1992; Schlosshauer, 1993) is formed by a monolayer of tightly connected microvascular endothelial cells with anionic charges. This layer separates two fluid-containing compartments: the blood plasma (BP) and extracellular fluid (ECF) of the brain parenchyma, and is surrounded by astroglial cells of the brain. One of the main functions of the BBB is to regulate the transfer of components between the BP and the ECF. The BBB limits free passage of most agent molecules from the blood to the brain cells.

[0023] In general, large molecules of high polarity, such as peptides, proteins, (e.g., enzymes, growth factors and their conjugates, oligonucleotides, genetic vectors and others) do not cross the BBB. Therefore poor agent delivery to the CNS limits the applicability of such macromolecules for the treatment of neurodegenerative disorders and neurological diseases.

[0024] Several delivery approaches of therapeutic agents to the brain circumvent the BBB. Such approaches utilize intrathecal injections, surgical implants (Ommaya, 1984 and U.S. Pat. No. 5,222,982) and interstitial infusion (Bobo et al., 1994). These strategies deliver an agent to the CNS by direct administration into the cerebrospinal fluid (CSF) or into the brain parenchyma (ECF).

[0025] Drug delivery to the central nervous system through the cerebrospinal fluid is achieved by means of a subdurally implantable device named after its inventor, the "Ommaya reservoir". The reservoir is used mostly for localized post-operative delivery of chemotherapeutic agents in cancers. The drug is injected into the device and subsequently released into the cerebrospinal fluid surrounding the brain. It can be directed toward specific areas of exposed brain tissue which then adsorb the drug. This adsorption is limited since the drug does not travel freely. A modified device developed by

Ayub Ommaya, whereby the reservoir is implanted in the abdominal cavity and the injected drug is transported by cerebrospinal fluid (taken from and returned to the spine) all the way to the ventricular space of the brain, is used for agent administration.

[0026] Diffusion of macromolecules to various areas of the brain by convection-enhanced delivery is another method of administration circumventing the BBB. This method involves: a) creating a pressure gradient during interstitial infusion into white matter to generate increased flow through the brain interstitium (convection supplementing simple diffusion); b) maintaining the pressure gradient over a lengthy period of time (24 hours to 48 hours) to allow radial penetration of the migrating compounds (such as: neurotrophic factors, antibodies, growth factors, genetic vectors, enzymes, etc.) into the gray matter; and c) increasing drug concentrations by orders of magnitude over systemic levels. Through their direct infusion into the brain parenchyma, the site-specific biomolecular complexes of U.S. Patent 6,005,004 deliver the agent to neuronal or glial cells, as needed, and be retained by these cells. Moreover, the site-specific complexes containing neuronal targeting or internalization moieties are capable of penetrating the neuronal membrane and internalizing the agent.

[0027] Another strategy to improve agent delivery to the CNS is by increasing the agent absorption (adsorption and transport) through the BBB and their uptake by the cells (Broadwell, 1989; Pardridge et al., 1990; Banks et al., 1992; and Pardridge, edited by Vranic et al., 1991. The passage of agents through the BBB to the brain can be enhanced by improving either the permeability of the agent itself or by altering the characteristics of the BBB. Thus, the passage of the agent can be facilitated by increasing its lipid solubility through chemical modification, and/or by its coupling to a cationic carrier, or still by its covalent coupling to a peptide vector capable of transporting the agent through the BBB. Peptide transport vectors are also known as BBB permeabilizer compounds (U.S. Pat. No. 5,268,164).

Phage Display:

[0028] Combinatorial phage display peptide libraries provide an effective means to study protein:protein interactions. This technology relies on the production of very large collections of random peptides associated with their corresponding genetic blueprints (Scott et al, 1990; Dower, 1992; Lane et al, 1993; Cortese et al, 1994; Cortese et al, 1995; Cortese et al, 1996). Presentation of the random peptides is often accomplished by constructing chimeric

proteins expressed on the outer surface of filamentous bacteriophages such as M13, fd and f1. This presentation makes the repertoires amenable to binding assays and specialized screening schemes (referred to as biopanning (Parmley et al, 1988)) leading to the affinity isolation and identification of peptides with desired binding properties. In this way peptides that bind to receptors (Koivunen et al, 1995; Wrighton et al, 1996; Sparks et al, 1994; Rasqualini et al, 1996), enzymes (Matthews et al, 1993; Schmitz et al, 1996) or antibodies (Scott et al, 1990; Cwirla et al, 1990; Felici et al, 1991; Luzzago et al, 1993; Hoess et al, 1993; Bonnycastle et al, 1996) have been efficiently selected.

[0029] Filamentous bacteriophages are nonlytic, male specific bacteriophages that infect *Escherichia coli* cells carrying an F-episome (for review, see Model et al, 1988). Filamentous phage particles appear as thin tubular structures 900 nm long and 10 nm thick containing a circular single stranded DNA genome (the + strand). The life cycle of the phage entails binding of the phage to the F-pilus of the bacterium followed by entry of the single stranded DNA genome into the host. The circular single stranded DNA is recognized by the host replication machinery and the synthesis of the complementary second DNA strand is initiated at the phage ori(-) structure. The double stranded DNA replicating form is

the template for the synthesis of single-stranded DNA circular phage genomes, initiating at the ori(+) structure. These are ultimately packaged into virions and the phage particles are extruded from the bacterium without causing lysis or apparent damage to the host.

[0030] Peptide display systems have exploited two structural proteins of the phage; pIII protein and pVIII protein. The pIII protein exists in 5 copies per phage and is found exclusively at one tip of the virion (Goldsmith et al, 1977). The N-terminal domain of the pIII protein forms a knob-like structure that is required for the infectivity process (Gray et al, 1981). It enables the adsorption of the phage to the tip of the F-pilus and subsequently the penetration and translocation of the single stranded phage DNA into the bacterial host cell (Holliger et al, 1997). The pIII protein can tolerate extensive modifications and thus has been used to express peptides at its N-terminus. The foreign peptides have been up to 65 amino acid residues long (Bluthner et al, 1996; Kay et al, 1993) and in some instances even as large as full-length proteins (McCafferty et al, 1990; McCafferty et al, 1992) without markedly affecting pIII function.

[0031] The cylindrical protein envelope surrounding the single stranded phage DNA is composed of 2700 copies of the

major coat protein, pVIII, an α -helical subunit which consists of 50 amino acid residues. The pVIII proteins themselves are arranged in a helical pattern, with the α -helix of the protein oriented at a shallow angle to the long axis of the virion (Marvin et al, 1994). The primary structure of this protein contains three separate domains: (1) the N-terminal part, enriched with acidic amino acids and exposed to the outside environment; (2) a central hydrophobic domain responsible for: (i) subunit:subunit interactions in the phage particle and (ii) transmembrane functions in the host cell; and (3) the third domain containing basic amino acids, clustered at the C-terminus, which is buried in the interior of the phage and is associated with the phage-DNA. pVIII is synthesized as a precoat protein containing a 23 amino acid leader-peptide, which is cleaved upon translocation across the inner membrane of the bacterium to yield the mature 50-residue transmembrane protein (Sugimoto et al, 1977). Use of pVIII as a display scaffold is hindered by the fact that it can tolerate the addition of peptides no longer than 6 residues at its N-terminus (Greenwood et al, 1991; Iannolo et al, 1995). Larger inserts interfere with phage assembly. Introduction of larger peptides, however, is possible in systems where mosaic phages are produced by *in vivo* mixing the recombinant, peptide-containing, pVIII proteins with wild type pVIII (Felici et al,

1991; Greenwood et al, 1991; Willis et al, 1993). This enables the incorporation of the chimeric pVIII proteins at low density (tens to hundreds of copies per particle) on the phage surface interspersed with wild type coat proteins during the assembly of phage particles. Two systems have been used that enable the generation of mosaic phages; the "type 8+8" and "type 88" systems as designated by Smith (Smith, 1993).

[0032] The "type 8+8" system is based on having the two pVIII genes situated separately in two different genetic units (Felici et al, 1991; Greenwood et al, 1991; Willis et al, 1993). The recombinant pVIII gene is located on a phagemid, a plasmid that contains, in addition to its own origin of replication, the phage origins of replication and packaging signal. The wild type pVIII protein is supplied by superinfecting phagemid-harboring bacteria with a helper phage. In addition, the helper phage provides the phage replication and assembly machinery that package both the phagemid and the helper genomes into virions. Therefore, two types of particles are secreted by such bacteria, helper and phagemid, both of which incorporate a mixture of recombinant and wild type pVIII proteins.

[0033] The "type 88" system benefits by containing the two pVIII genes in one and the same infectious phage genome. Thus, this obviates the need for a helper phage and

superinfection. Furthermore, only one type of mosaic phage is produced.

[0034] The phage genome encodes 10 proteins (pI through pX) all of which are essential for production of infectious progeny (Felici et al, 1991). The genes for the proteins are organized in two tightly packed transcriptional units separated by two non-coding regions (Van Wezenbeek et al, 1980). One non-coding region, called the "intergenic region" (defined as situated between the pIV and pII genes) contains the (+) and the (-) origins of DNA replication and the packaging signal of the phage, enabling the initiation of capsid formation. Parts of this intergenic region are dispensable (Kim et al, 1981; Dotto et al, 1984). Moreover, this region has been found to be able to tolerate the insertion of foreign DNAs at several sites (Messing, 1983; Moses et al, 1980; Zacher et al, 1980). The second non-coding region of the phage is located between the pVIII and pIII genes, and has also been used to incorporate foreign recombinant genes as was illustrated by Pluckthun (Krebber et al, 1995).

In vivo Imaging:

[0035] The use of contrast agents in diagnostic medicine is rapidly growing. In X-ray diagnostics, for example, increased

contrast of internal organs, such as the kidneys, the urinary tract, the digestive tract, the vascular system of the heart (angiography), and so forth is obtained by administering a contrast agent which is substantially radiopaque. In conventional proton MRI diagnostics, increased contrast of internal organs and tissues may be obtained by administering compositions containing paramagnetic metal species which increase the relaxation rate of surrounding protons. In ultrasound diagnostics, improved contrast is obtained by administering compositions having acoustic impedances different than that of blood or other tissues.

[0036] MRI encompasses the detection of certain atomic nuclei utilizing magnetic fields and radio-frequency radiation is now well established as a medical diagnostic tool. It is similar in some respects to X-ray computed tomography (CT) in providing a cross-sectional display of the body organ anatomy with excellent resolution of soft tissue detail. As currently used, the images produced constitute a map of the proton density distribution, the relaxation times, or both, in organs and tissues. The technique of MRI is advantageously non-invasive as it avoids the use of ionizing radiation.

[0037] While the phenomenon of NMR was discovered in 1945, it is only recently that it has found application as a means of mapping the internal structure of the body as a result of

the original suggestion of Lauterbur, 1973. The fundamental lack of any known hazard associated with the level of magnetic and radio-frequency fields that are employed renders it possible to make repeated scans on vulnerable individuals. In addition to standard scan planes (axial, coronal, and sagittal), oblique scan planes can also be selected.

[0038] With an MRI experiment, the nuclei under study in a sample (e.g. protons) are irradiated with the appropriate radio-frequency (RF) energy in a highly uniform magnetic field. These nuclei, as they relax, subsequently emit RF at a sharp resonance frequency. The resonance frequency of the nuclei depends on the applied magnetic field.

[0039] According to known principles, nuclei with appropriate spin when placed in an applied magnetic field (B , expressed generally in units of gauss or Tesla [10^4 gauss]) align in the direction of the field. In the case of protons, these nuclei precess at a frequency, f , of 42.6 MHz, at a field strength of 1 Tesla. At this frequency, an RF pulse of radiation will excite the nuclei and can be considered to tip the net magnetization of the field direction, the extent of this rotation being determined by the pulse duration and energy. After the RF pulse, the nuclei "relax" or return to equilibrium with the magnetic field, emitting radiation at

the resonant frequency. The decay of the emitted radiation characterized by two relaxation times, i.e., T_1 , the spin-lattice relaxation time or longitudinal relaxation time, that is, the time taken by the nuclei to return to equilibrium along the direction the externally applied magnetic field, and T_2 , the spin-spin relaxation time associated with the dephasing of the initially coherent precession of individual proton spins. These relaxation times have been established for various fluids, organs and tissues in different species of mammals.

[0040] In MRI, scanning planes and slice thicknesses can be selected. This selection permits high quality transverse, coronal and sagittal images to be obtained directly. The absence of any moving parts in MRI equipment promotes high reliability. It is believed that MRI has a greater potential than CT for the selective examination of tissue characteristics in view of the fact that in CT, X-ray attenuation coefficients alone determine image contrast, whereas at least five separate variables (T_1 , T_2 , proton density pulse sequence and flow) may contribute to the MRI signal.

[0041] By reason of its sensitivity to subtle physiochemical differences between organs and/or tissues, it is believed that MRI may be capable of differentiating

different tissue types in detecting diseases which induce physiochemical changes that may not be detected by X-ray or CT which are only sensitive to differences in the electron density of tissue.

[0042] As noted above, two of the principal imaging parameters are the relaxation times, T_1 and T_2 . For protons (or other appropriate nuclei), these relaxation times are influenced by the environment of the nuclei, (e.g., viscosity, temperature, and the like). These two relaxation phenomena are essentially mechanisms whereby the initially imparted radio-frequency energy is dissipated to the surrounding environment. The rate of this energy loss or relaxation can be influenced by certain other nuclei which are paramagnetic. Chemical compounds incorporating these paramagnetic nuclei may substantially alter the T_1 and T_2 values for nearby protons. The extent of the paramagnetic effect of a given chemical compound is a function of the environment.

[0043] The majority of materials now being proposed as MRI contrast media achieve a contrast effect because they contain paramagnetic, superparamagnetic or ferromagnetic species.

[0044] For ferromagnetic and superparamagnetic contrast agents, which are negative MRI contrast agents, the enhanced image contrast derives primarily from the reduction in the spin reequilibration parameter known as arising from the

effect on the imaging nuclei of the fields generated by the ferromagnetic or superparamagnetic particles.

[0045] Paramagnetic contrast agents on the other hand may be either positive or negative MRI contrast agents. The effect of paramagnetic substances on magnetic resonance signal intensities is dependent on many factors, the most important of which are the concentration of the paramagnetic substances at the imaged site, the nature of the paramagnetic substance itself, and the pulse sequence and magnetic field strength used in the imaging routine.

[0046] Generally, however, paramagnetic contrast agents are positive MRI contrast agents at low concentrations where their T_1 lowering effect dominates, and negative MRI contrast agents at higher concentrations where their T_2 lowering effect is dominant. In either event, the relaxation time reduction results from the effect on the imaging nuclei of the magnetic fields generated by the paramagnetic centers.

[0047] The use of paramagnetic, ferromagnetic and superparamagnetic materials as MRI contrast agents has been widely advocated, and broad ranges of suitable materials have been suggested in the literature.

[0048] In general, paramagnetic species such as ions of elements with atomic numbers of 21 to 29, 42 to 44 and 58 to 70 have been found effective as MRI contrasting agents.

Examples of suitable ions include chromium(III), manganese(II), manganese(III), iron(II), iron(III), cobalt(II), suitable ions include chromium(III), manganese(II), manganese(III), iron(II), iron(III), cobalt(II), nickel(II), copper(II), praseodymium(III), neodymium(III), samarium(III), and ytterbium(III). Because of their very strong magnetic moments, gadolinium(III), terbium(III), dysprosium(III), holmium(III) and erbium(III) are preferred. Gadolinium(III) ions have been particularly preferred as MRI contrasting agents.

[0049] It might seem that the aqua ion of each of these paramagnetic metals would be a good choice for use as a contrast agent, as these have the largest possible number of bound water molecules. However, the aqua ions are relatively toxic, and there exists little opportunity to control the biodistribution of these species. The reported LD₅₀ values for the metal chloride salts in aqueous solution are 1.4, 1.5 and 1.6 mmol/kg for gadolinium, manganese and iron respectively when administered to mice i.p. See, Lauffer, 1987.

[0050] In attempts to solve both of these problems, a variety of organic complexing/chelating agents--organic molecules which are able to coordinate to the metal ions--have been employed. For current clinical contrast agents that are based on gadolinium, complexing/chelating agents are employed

which occupy almost all of the coordination sites on the metal ion, typically leaving one site available for water molecules to reversibly bind. This approach reduces the toxicity of the metal ion and, by careful variation of the complexing/chelating system, potentially allows control of the biodistribution such that *in vivo* targeting may be achieved. Other desirable properties of a potential contrast agent may include prompt clearance of an extracellular agent as well as *in vivo* and *in vitro* stability.

[0051] Typically, paramagnetic ions have been administered in the form of complexes with organic complexing agents. Such complexes provide the paramagnetic ions in a soluble, non-toxic form, and facilitate their rapid clearance from the body following the imaging procedure. Gries et al., U.S. Pat. No. 4,647,447, disclose complexes of various paramagnetic ions with conventional aminocarboxylic acid complexing agents. A preferred complex disclosed by Gries et al. is the complex of gadolinium(III) with diethylenetriamine-pentaacetic acid ("DTPA"). Paramagnetic ions, such as gadolinium(III), have been found to form strong complexes with DTPA, ethylenediamine-tetraacetic acid ("EDTA"), and with tetraazacyclododecane-N,N', N'',N'''-tetraacetic acid ("DOTA").

[0052] These complexes do not dissociate substantially in physiological aqueous fluids. The gadolinium complex of DTPA

has a net charge of -2, whereas the gadolinium complex of EDTA or DOTA has a net charge of -1, and both are generally administered as soluble salts. Typical salts are sodium and N-methylglucamine. The administration of salt is attended by certain disadvantages. These salts can raise the *in vivo* ion concentration and cause localized disturbances in osmolality, which in turn, can lead to edema and other undesirable reactions.

[0053] Efforts have been made to design new ionic and neutral paramagnetic metal complexes which avoid or minimize the above mentioned disadvantages. In general, this goal can be achieved by converting one or more of the free carboxylic acid groups of the complexing agents to neutral, non-ionizable groups. For example, S. C. Quay, in U.S. Patent 4,687,658 and 4,687,659, discloses alkylester and alkylamide derivatives, respectively, of DTPA complexes. Similarly, Dean et al., U.S. Patent 4,826,673 discloses mono- and polyhydroxy-alkylamide derivatives of DTPA and their use as complexing agents for paramagnetic ions. It can also be achieved by covalent attachment of organic cations of the complexing agent in such a manner that the sum of positive and negative charges in the resulting metal complex is zero.

[0054] Citation of any document herein is not intended as an admission that such document is pertinent prior art, or

considered material to the patentability of any claim of the present application. Any statement as to content or a date of any document is based on the information available to applicant at the time of filing and does not constitute an admission as to the correctness of such a statement.

SUMMARY OF THE INVENTION

[0055] According to one aspect of the present invention there is provided a method of treating a plaque forming disease comprising the steps of (a) displaying a polypeptide on a display vehicle, the polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable of eliciting antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein; and (b) introducing the display vehicle into a body of a recipient so as to elicit the antibodies capable of disaggregating the aggregating protein and/or of preventing or inhibiting aggregation of the aggregating protein.

[0056] According to another aspect of the present invention there is provided an agent for treating a plaque forming disease comprising a display vehicle displaying a polypeptide representing at least one epitope of an aggregating protein

associated with plaque formation in the plaque forming disease, the at least one epitope being capable of eliciting antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein.

[0057] According to yet another aspect of the present invention there is provided a pharmaceutical composition for treating a plaque forming disease comprising an effective amount of a display vehicle displaying a polypeptide, the polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable of eliciting an effective amount of antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein, the pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

[0058] According to still another aspect of the present invention there is provided a method of preparing a display vehicle for treating a plaque forming disease, the method comprising the step of genetically modifying a genome of a display vehicle by inserting therein a polynucleotide sequence encoding a polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable

of eliciting antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein, such that when the display vehicle propagates the polypeptide is displayed by the display vehicle.

[0059] According to an additional aspect of the present invention there is provided a method of treating a plaque forming disease comprising the steps of (a) displaying a polypeptide representing at least an immunological portion of an antibody being for binding at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the binding capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein; and (b) introducing the display vehicle into a body of a recipient so as to disaggregate the aggregating protein and/or prevent its aggregation.

[0060] A further aspect of the present invention provides a method of treating a neurological disease or disorder of the CNS that involves displaying a therapeutic molecule capable of treating the neurological disease or disorder on a viral display vehicle and introducing the viral display vehicle into a subject in need thereof by applying an effective amount of the viral display vehicle displaying the therapeutic molecule to an olfactory system of the subject to treat a neurological

disease or disorder that is plaque-forming or that is non-plaque-forming.

[0061] An additional aspect of the present invention relates to a pharmaceutical composition for treating a neurological disease or disorder of the CNS which includes a pharmaceutically acceptable carrier and an effective amount of a viral display vehicle displaying a therapeutic molecule capable of treating a neurological disease or disorder of the CNS.

[0062] A still further aspect of the present invention provides a method of diagnosing the presence or extent of a neurological disease or disorder of the CNS by *in vivo* imaging. This diagnostic method involves displaying on a viral display vehicle a diagnostic agent capable of being detected by *in vivo* imaging, introducing the viral display vehicle into a subject by applying the viral display vehicle displaying the diagnostic agent to an olfactory system of the subject, and detecting the displayed diagnostic agent in the subject by *in vivo* imaging to diagnose the presence or extent of the neurological disease or disorder.

[0063] Yet a further aspect of the present invention relates to a pharmaceutical composition for diagnosing the presence or extent of a neurological disease or disorder of the CNS which includes a pharmaceutically acceptable carrier

and an effective amount of a viral display vehicle which displays a targeting agent and a diagnostic agent capable of being detected by *in vivo* imaging.

[0064] According to still an additional aspect of the present invention there is provided a method of introducing a display vehicle lacking an engineered targeting moiety into a brain of a recipient, the method comprising the step of administering the display vehicle intranasally to the recipient.

[0065] According to further features in preferred embodiments of the invention described below, the step of introducing the display vehicle into the body of the recipient so as to disaggregate the aggregating protein is effected through an olfactory system of the recipient.

[0066] According to yet another additional aspect of the present invention there is provided an agent for treating a plaque forming disease comprising a display vehicle displaying a polypeptide representing at least an immunological portion of an antibody which can bind at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the immunological portion of the antibody being capable of disaggregating said aggregating protein and/or of preventing aggregation of the aggregating protein.

[0067] According to yet an additional aspect of the present invention there is provided a pharmaceutical composition for treating a plaque forming disease comprising an effective amount of a display vehicle displaying a polypeptide representing at least an immunological portion of an antibody which can bind at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the immunological portion of the antibody being capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein, the pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

[0068] According to still an additional aspect of the present invention there is provided a method of preparing a display vehicle for treating a plaque forming disease comprising the step of genetically modifying a genome of a display vehicle by inserting therein a polynucleotide sequence encoding at least an immunological portion of an antibody capable of binding at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the immunological portion of the antibody being capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein.

[0069] According to a further aspect of the present invention there is provided a polypeptide comprising at least an immunological portion of an antibody being capable disaggregating a prion protein aggregate and/or of preventing aggregation of said prion protein.

[0070] According to further features in preferred embodiments of the invention described below, the polypeptide is capable of binding at least one epitope formed by an amino acid sequence set forth in SEQ ID NO:25.

[0071] According to yet a further aspect of the present invention there is provided a method of detecting a presence or an absence of a prion protein in a biological sample, the method comprising the steps of: (a) incubating an anti-prion antibody or an immunological portion thereof with the biological sample; (b) determining a presence or an absence of antigen complexes formed with the anti-prion antibody or the immunological portion thereof, to thereby determine the presence or the absence of the prion protein in the biological sample.

[0072] According to still further features in the described preferred embodiments the plaque forming disease is selected from the group consisting of early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's

disease, SAA amyloidosis, hereditary Icelandic syndrome, senility and multiple myeloma.

[0073] According to still further features in the described preferred embodiments the plaque forming disease is selected from the group consisting of scrapie, bovine spongiform encephalopathy (BSE), kuru, Creutzfeldt-Jakob Disease (CJD), Gerstmann-Streussler-Sheinker Disease (GSS) and fatal familial insomnia (FFI).

[0074] According to still further features in the described preferred embodiments the aggregating protein is selected from the group consisting of beta-amyloid, serum amyloid A, cystatin C, IgG kappa light chain and prion protein.

[0075] According to still further features in the described preferred embodiments the display vehicle is selected from the group consisting of a virus, a bacteria and a polypeptide carrier.

[0076] According to still further features in the described preferred embodiments the virus is selected from the group consisting of a double stranded DNA virus, a single stranded DNA virus, a positive strand RNA virus and a negative strand RNA virus.

[0077] According to still further features in the described preferred embodiments the display vehicle is a bacteriophage.

[0078] According to still further features in the described preferred embodiments the display vehicle is a filamentous bacteriophage.

[0079] According to still further features in the described preferred embodiments the bacteriophage display vehicle is capable of propagating within bacterial flora of the host.

[0080] According to still further features in the described preferred embodiments the bacteriophage display vehicle is capable of propagating within *E. coli*.

[0081] According to still further features in the described preferred embodiments the bacteriophage display vehicle is fd.

[0082] According to further features in the described preferred embodiments of the invention, the display vehicle is incapable of propagation *in vivo*.

[0083] According to still further features in the described preferred embodiments a triple dose of 10^{10} units of the chosen display vehicle induces an antibody titer of at least 1:50,000 within 30 days of administration, as measured by ELISA.

[0084] According to still further features in the described preferred embodiments the at least one epitope of said prion protein is formed by an amino acid sequence set forth in SEQ ID NO:25.

[0085] According to still further features in the described preferred embodiments the immunological portion of an antibody serves for binding at least one epitope of an aggregating protein associated with plaque formation in a plaque forming disease, said immunological portion of said antibody being capable of disaggregating said aggregating protein and/or of preventing aggregation of said aggregating protein.

[0086] According to still further features in the described preferred embodiments the prion protein is the aggregating protein associated with plaque formation

[0087] According to still further features in the described preferred embodiments the biological sample is derived from tissues and/or body fluids of a human, a primate, a monkey, a pig, a bovine, a sheep, a deer, an elk, a cat, a dog and a chicken.

[0088] The present invention successfully addresses the shortcomings of the presently known configurations by providing methods, agents, and pharmaceutical compositions for preventing or reversing the progression of a plaque forming disease. The present invention further includes methods for preparing agents and pharmaceutical compositions useful for preventing or treating plaque forming diseases and to a method of detecting the presence of a pathogenic prion protein in a biological sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0089] The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

[0090] FIG. 1A is a schematic depiction of an IgM antibody.

[0091] FIG. 1B is a photograph of an ethidium bromide stained 1.5 % agarose gel showing cDNA fragments of the heavy and the light chains of IgM508. Lane 1: Kb (Ladder); Lanes 2 and 3 V_H and V_L fragments, respectively, as indicated by arrows.

[0092] FIG. 1C is a photograph of an ethidium bromide stained 1.5 % agarose gel showing scFv DNA fragment derived from antibody IgM 508. Lane 1: Kb (Ladder); Lane 2: scFv 508 DNA (750 bp).

[0093] FIG. 1D is a schematic depiction of filamentous phage displaying an scFv.

[0094] FIG. 1E is a schematic depiction of a soluble scFv.

[0095] FIG. 2 is a physical map of plasmid pCC-508F which is used for the production of scFv-508-CBD fusion protein (also referred to herein as 508(Fv)-CBD) under control of lac promoter. Amp res - a gene encoding β -lactamase; V_H and V_L - sequences coding for the variable domains of the heavy and light chains of scFv-508, respectively; Lin - a gene coding for a (Gly₄Ser)₃ (SEQ ID NO:2) linker present between the variable domains V_H and V_L . Restriction sites and positions thereof are also shown.

[0096] FIG. 3 is a physical map of plasmid pFFEKCA-508 which is used according to the present invention for cytoplasmic expression of the scFv-508-CBD fusion protein under the control of a T₇-promoter. Amp res - a gene encoding β -lactamase; V_H and V_L - sequences coding for the variable domains of the heavy and light chains of scFv-508, respectively; Lin - a gene coding for a (Gly₄Ser)₃ (SEQ ID

NO:2) linker present between the variable domains V_H and V_L . T7-promoter and T7 term - T7 promoter and T7 terminator sequences, respectively. Restriction sites and positions thereof are also shown.

[0097] FIG. 4 shows an analysis of β AP binding by antibody 508(Fv)-CBD in an ELISA assay. The analyzed antibodies were added to β AP coated wells. Bound antibodies were detected with HRP conjugated secondary antibodies. The parental 508 IgM antibody was used as a positive control. The unrelated anti- β -galactosidase antibody Gal6(Fv)-CBD was used as a negative control.

[0098] FIG. 5 shows PCR analysis of phage DNA inserts.. DNA isolated from pCC-508(Fv), lane 2, and pCC-Gal6(Fv), Lane 3, were PCR amplified and separated on a 1.5 % agarose gel. Ethidium bromide staining and UV illumination were used to visualize the bands. Lane 1 contains a DNA size marker. The arrow marks the position of an intact scFv migrating at about 750 bp.

[0099] FIG. 6 demonstrates expression and purification of 508(Fv)-CBD. 5-10 μ g protein were loaded in each lane of a 14 % SDS polyacrylamide gel. Proteins were visualized by Coomassie brilliant blue staining. The arrow marks the position of the scFv-CBD fusion protein. Lane 1 - total cell extract from non-induced BL21(DE3) cells carrying 508((Fv)-CBD

expression vector. Lane 2 - total cell extract from BL21(DE3) cells carrying 508(Fv)-CBD expression vector induced for 3 hours with IPTG. Lane 3 - washed, solubilized and reduced inclusion bodies that were used in refolding. Lane 4 - protein that did not bind to cellulose during cellulose-assisted refolding. Lane 5 - protein washed away from cellulose with TBS. Lane 6 - protein washed away from crystalline cellulose with distilled water. Lane 7 - soluble 508(Fv)-CBD recovered from cellulose by high-pH elution and neutralization.

[00100] FIG. 7 demonstrates the stability of 508(Fv)-CBD. Purified 508(Fv)-CBD protein was stored at 4 °C for one day (dark squares) or one week (dark circles), and then analyzed for β AP binding in an ELISA assay, as described in the legend to Figure 4. The unrelated antibody Gal6(Fv)-CBD served as a negative control (open squares).

[00101] FIG. 8 demonstrates quantitation of 508(Fv) mutants affinity-enrichment by PCR and DNA restriction analysis. The DNA of 19 508(Fv)-mutant micro library clones before (Figure 8a) and of 11 clones picked up after one cycle of affinity selection (Figure 8b) were analyzed. The DNA was digested with *PvuI* and separated on a 1.5 % agarose gel. A non-mutated scFv-CBD appears as an intact 1250 bp fragment (upper arrow). A mutated clone is indicated by the appearance of both 700 bp

(middle arrow) and 550 bp (lower arrow) fragments. A DNA size marker is shown in lane 1.

[00102] FIG. 9 shows an analysis of β AP binding (Figure 9a) and stability (Figure 9b) of mutated 508(Fv) derivatives in an ELISA assay. The analyzed antibodies were added to β AP coated wells. Bound antibodies stored at 4 °C for one day or for one week were detected as described in the legend to Figure 7. 508(Fv) wild type (open squares), C96F (dark squares), C96Y (dark circles), C96S (dark triangles). The unrelated anti- β -galactosidase antibody Gal6(Fv)-CBD was used as a negative control (open squares).

[00103] FIG. 10 shows an analysis of the specific inhibition of β AP binding by antibody 508F(Fv) in a competitive ELISA assay. The antibody was pre-incubated with varying concentrations of the competing peptides: β AP (acids 1-16 of SEQ ID NO:3) (dark squares) or the unrelated peptide WULD (SEQ ID NO:4) (open squares), before being added to β AP coated wells. Bound antibodies were detected as described in the legend to Figure 7.

[00104] FIGS. 11A and 11B show nucleotide (SEQ ID NO:5) and deduced amino acid (SEQ ID NO:6) sequences of scFv 508F heavy chain (Figure 11A); and the linker and the variable region of the light chain (Figure 11B) (SEQ ID NOS:27-28). The amino

acid sequence is presented by a three-letter code; CDRs and the linker are underlined.

[00105] FIG. 12 demonstrates the prevention of β AP mediated toxic effect on PC12 cells by 508F(Fv). Cells were incubated with fibrillar β A alone, or with fibrillar β A that had been incubated with antibodies at different molar ratio of antibody/ β AP, as indicated. An 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to estimate cell survival.

[00106] FIG. 13 demonstrates the disaggregation of fibrillar β A by 508F(Fv). The fibrillar state of pre-formed β A fibrils were measured with or without incubation with antibodies at different molar ratio of antibody/ β AP, as indicated. The fluorescence of thioflavin-T (ThT) reagent in a ThT assay which is proportional to fibril β A was used to assess the fibril morphology.

[00107] FIGS. 14A-D demonstrate the detection of filamentous phage (f88-EFRH) in brain sections via immunofluorescence one day following a single dose applied intranasally. Appearance of filamentous phage in mouse olfactory bulb and hippocampus sections using fluorescent rabbit anti-phage antibody (Figures 14A and 14C, respectively) as is compare to an untreated mouse brain (Figures 14B and 14D, respectively). The sections were

observed using a fluorescence microscope at a final magnification of $\times 10$.

[00108] FIGs. 15A-D demonstrate the disappearance of filamentous phage (f88-EFRH) from mouse brain 28 days following a single intranasal administration. Disappearance of filamentous phage from mouse olfactory bulb and hippocampus is demonstrated in sections of these organs using fluorescent rabbit anti-phage antibody (Figures 15A and 15C, respectively), as is compared to an untreated mouse brain (Figures 15B and 15D, respectively). The sections were observed using a fluorescence microscope at a final magnification of $\times 10$.

[00109] FIGs. 16A-D show histology of mouse brain sections after phage f88-EFRH clearance. Brain sections of olfactory organ (Figure 16A) and hippocampus (Figure 16C) after 28 days following phage f88-EFRH administration were stained with hematoxylin and eosin, and compared to sections of an untreated brain (Figures 16B and 16D, respectively). The stained sections were examined and photographed at a final magnification of $\times 40$.

[00110] FIGs. 17A-D show fluorescence detection of biotin of phage pCC-508F coupled to biotinylated β AP (acids 1-16 of SEQ ID NO:3) in mouse brain sections following a single intranasal administration. Appearance of β AP (acids 1-16, SEQ ID NO:3)

coupled to filamentous phage displaying scFv508F in mice olfactory bulb and hippocampus sections using streptavidin coupled to PE (Figures 17A and 17C, respectively) as is compare to an untreated mouse brain (Figures 17B and 17D, respectively). The sections were observed using a fluorescence microscope at a final magnification of x 20.

[00111] FIGS. 18A-D show histology of mouse brain after phage pCC-508F coupled to biotinylated β AP (acids 1-16 of SEQ ID NO:3) administration. Olfactory organ (Figure 18A) and hippocampus (Figure 18B) sections one day following phage administration were stained with hematoxylin and eosin, and were compared to untreated mouse brain sections (Figures 18C and 18D, respectively). The stained sections were examined and photographed at a final magnification of x 40.

[00112] FIG. 19 is a diagram of immunization schedule with filamentous phage displaying the EFRH (SEQ ID NO:1) epitope of β -amyloid peptide.

[00113] FIGS. 20A and 20B show immunization with f3 filamentous phage displaying EFRH (SEQ ID NO:1) epitope of β -amyloid peptide as a fusion of phage glycoprotein III (gpIII). Serum IgG titer of different bleeds from mice immunized with the EFRH-phage according to the schedule of Figure 19 against wild type filamentous phage coat proteins (Figure 20A) and the

N-terminal epitope (acids 1-16, SEQ ID NO:3) of β -amyloid (Figure 20B).

[00114] FIG. 21 demonstrates long lasting immunization with f3 filamentous phage. Serum IgG titer of different bleeds from mice immunized with EFRH-phage against wild type filamentous phage coat proteins and the N-terminal (acids 1-16, SEQ ID NO:3) of β -amyloid.

[00115] FIG. 22 show binding of anti-aggregating β AP monoclonal antibody (mAb 10D5) to peptide-presenting phage selected from an f88 phage library. Unrelated mAb 5.5 raised against acetylcholine receptor was used as a negative control. Antibodies were added to phage-coated wells and ELISA was used to detect binding.

[00116] FIG. 23 show binding of anti-aggregating β AP mAb (10D5) to a YYEFRH (SEQ ID NO:7)-phage and VHEPHEFRHVALNPV (SEQ ID NO:8)-phage. Antibody in concentration of 1 μ g/ml was added to phage-coated wells and binding was analyzed by ELISA. Filamentous phage without insert was used as a control.

[00117] FIGs. 24A-B show immunization with f88 filamentous phage displaying EFRH (SEQ ID NO:1) epitope of β -amyloid peptide as a fusion of phage glycoprotein VIII (gpVIII). Serum IgG titer of different bleeds from mice immunized with EFRH-phage against wild type filamentous phage coat proteins

(Figure 24A) and the N-terminal epitope (acids 1-16, SEQ ID NO:3) of β -amyloid peptide (Figure 24B).

[00118] FIG. 25 shows inhibition of serum of an immunized mice in binding to β AP by synthetic peptides derived from the N-terminal of β -amyloid peptide. The assay was done with 1:3000 dilution of serum after a third immunization with f88-EFRH reacted with the various peptides in various concentrations per well, as indicated. The peptide WVLD (SEQ ID NO:4) was used as a negative control.

[00119] FIG. 26 demonstrates prevention of β AP mediated toxic effect on PC12 cells by serum antibodies raised against f88-EFRH-phage. Cells were incubated with fibrillar β A alone, or with fibrillar β A that has been incubated with serum from the third bleeding at different concentration. The negative control was serum from a non-immunized mouse. The MTT assay was used to estimate cell survival.

[00120] FIG. 27 demonstrates interference with fibrillar β -amyloid formation by serum antibodies raised against the f88-EFRH-phage. Estimation of the fluorescence of ThT which correlates with the amount of fibrillar β -amyloid formed after incubation for a week at 37 °C in the presence of serum samples diluted as indicated. The negative control was serum

from a non-immunized mouse. The positive control was without serum. Fibril formation was measured by the ThT assay.

[00121] FIG. 28 illustrates the amino acids sequence corresponding to the human prion protein 106-126 (SEQ ID NO:25) and to the mouse homologue (SEQ ID NO:29).

[00122] FIG. 29 demonstrates the neurotoxicity effect of the PrP peptide as measured by MTT assay. PC12 cells were seeded in 96 well plates in a DMEM medium supplemented with 2mM insulin, 2 μ M L-glutamine and 100 units penicillin/streptomycin. Cell viability was assessed by the MTT assay following incubation with PrP 106-126, (at different concentrations). PrP 106-126 was either preincubated for 4 days at 37°C and then added to the cells for 3 days (grey bars), or was preincubated for 4 days at 37°C and then added to the cells for 5 days (white bars) or was preincubated for 7 days at 37°C and was then added to the cells for 5 days (black bars).

[00123] FIG. 30 illustrates the extent of aggregation of the PrP peptide, using ThT binding assay. PrP 106-126 (0-0.8 mg/ml) was incubated for 7 days at 37 °C and emission at 482 nm was measured to determine the extent of aggregation.

[00124] FIG. 31 demonstrates the protective effect of mAbs 3-11, 2-40 on PrP peptide neurotoxicity. PC12 cells were seeded in a 96 wells plate in a DMEM medium supplemented with

2mM insulin 2mM L-glutamine and 100 units penicillin/streptomycin and were incubated for three days. The following treatments were conducted: (1) Positive Control, untreated cells; (2) 100 mM PrP 106-126 that was preincubated for 7 days at 37°C; (3,4,5) an aggregated peptide that was preincubated for 1 hour before exposure to the cells together with the mAbs 3-11 (treatment 3), 2-40 (treatment 4) and 3F4 (treatment 5). Cell viability was assessed using the MTT assay.

[00125] FIG. 32 illustrates the modulation of PrP conformation by the mAbs. PrP 106-126 (0.3 mg/ml) was incubated for 7 days at 37°C (1) and with mAbs 2-40, 3-11 and 3F4 (treatments 2, 3 and 4, respectively). The antibodies were incubated with the sample for 24 hours either prior to the PrP incubation (grey bars) or following a one week PrP incubation (white bars). Fibril formation was assessed by the ThT binding assay.

[00126] FIG. 33 shows the concentration dependent protective effect of mAb 3-11 against PrP fibrillar aggregate formation. PrP 106-126 (0.3 mg/ml) was incubated for 7 days at 37°C with diluted mAb 3-11 (1:1, 1:10, 1:50, corresponding to treatments 1, 2, and 3, respectively). The antibody was incubated with the sample for 24 hours either prior to (grey bars), or

following (white bars), the one week incubation of PrP.
Amyloid fibril formation was assessed using ThT binding assay

DETAILED DESCRIPTION OF THE INVENTION

[00127] The present invention is directed to methods and pharmaceutical compositions for treating and/or diagnosing the presence or extent of neurological diseases and disorders using a display vehicle for delivery of a therapeutic or diagnostic agent. The present invention is more specifically directed to methods, pharmaceutical agents and compositions, which can be used for treating and diagnosing plaque-forming diseases, including, but not limited to, Alzheimer's disease and prion generated plaque forming diseases. Specifically the present invention can be used to (i) induce active immunity to plaque derived antigens in a recipient by immunizing with at least one epitope of an aggregating protein associated with plaque formation in a plaque forming disease on a display vehicle, so that antibodies elicited in response to immunization are capable of preventing plaque formation and/or of disaggregating existing plaques; and (ii) induce passive immunity by administering at least an immunological portion of an antibody which can bind to at least one epitope of an aggregating protein associated with plaque formation in a plaque forming disease, raised against plaque derived

antigens, cloned and displayed on a display vehicle, capable of preventing plaque formation and of disaggregating existing plaques. This passive immunity may be of exceptionally long duration if the display vehicle employed is capable of replicating within the recipient. The present invention further relates to a method of targeting a display vehicle to the brain of an animal, including man, so that plaques present in the brain, such as beta amyloid plaques in brains of Alzheimer's disease patients, may be disaggregated. Finally, the present invention also related to a method of detecting aggregate forming prion proteins in a biological sample.

[00128] The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

[00129] Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

[00130] While reducing one aspect of the present invention to practice, as is further exemplified in Examples 1-15 of the Examples section that follows, antigens derived from beta amyloid peptide were displayed on the surface of a filamentous phage which was used for immunization of experimental animals. All of the peptides employed contained the EFRH epitope (SEQ ID NO:1, corresponding to residues 3-6 of SEQ ID NO:3) of beta amyloid peptide (SEQ ID NO:3). The epitope was presented as a fusion protein of fd phage coat glycoprotein III or VIII. Doses ranging from 10^{10} to 10^{12} phages per injection were employed on 8 week old female BALB/c mice. A typical immunization schedule included three injections at 14-day intervals, administered either intraperitoneally or intranasally.

[00131] During and after the immunization process, the antibody serum titer of subject mice was tested for the production of A β specific antibodies by enzyme linked immunosorbent assay (ELISA) as detailed in the methods and materials section of the Examples hereinbelow. Serum titers were subsequently shown to persist for 11 months in response to a protocol including only 3 immunizations. While all tested epitopes containing EFRH produced a titer, displaying the epitope on the surface of a display vehicle produced far highest and unexpected titers. These high titers are believed

to be a result of the great number of copies presented to the immune system using this method, and this idea is supported by results of binding assays using controlled amounts of sera.

[00132] The anti-aggregating properties of the obtained polyclonal antibody raised against EFRH epitopes with respect to beta-amyloid fibril formation was measured by the ThT binding assay. Serum, at dilution of 1:10 and 1:100, disrupted formation of fibril structure of β -amyloid with extensive deterioration of fibril morphology, as indicated by a substantial decrease in ThT fluorescence. The unrelated serum used as control (serum from un-immunized mouse) did not significantly inhibit fibril formation.

[00133] The effect of the same serum on disruption of already formed β A fibril (the toxic form of β AP) was also determined. Serum of EFRH immunized mice incubated with pre-formed β A fibrils disrupted the fibril structure. The unrelated control antibody had no significant effect on fibril morphology. Together, these results confirm the ability of EFRH epitope presented by suitable display vehicles to evoke production of anti-aggregation antibodies, which can inhibit or reverse the process of fibril formation.

[00134] Diluted serum produced according to this embodiment of the present invention prevented the neurotoxicity of beta amyloid peptide. This result implies potential clinical

utility in preventing brain deterioration of patients suffering from amyloid plaque diseases.

[00135] While reducing another aspect of the present invention to practice, and as is further exemplified in Examples 15-21 of the Examples section which follows, it was uncovered that site-directed antibodies (designated mAbs 3-11 and 2-40), which were generated against a prion derived peptide, are useful in preventing or disaggregating prion generated plaques.

[00136] Binding of the prion derived peptide (PrP 106-126) to these mAbs led to a significant protective effect against aggregation as was measured by the ThT and MTT assays. The mAbs generated by the present invention also significantly decrease the peptide fibrillar aggregation and reverse the aggregated form to a disaggregated conformation as assayed by the ThT binding assay.

[00137] The binding of mAbs 3-11 and 2-40 to the PrP peptide either in solution or to the aggregate suggests that this epitope is involved in aggregation process and may act as a regulatory site controlling both the solubilization and disaggregation process of PrP peptide and perhaps the whole PrP protein.

[00138] Thus, according to one aspect of the present invention there is provided a method of treating a plaque

forming disease. The method according to this aspect of the present invention is effected by displaying a polypeptide on a display vehicle, the polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable of eliciting antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein, and introducing the display vehicle into a body of a recipient, so as to elicit the antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein.

[00139] According to a preferred embodiment of the present invention the display vehicle is selected such that less than 30 days following an introduction of a triple dose of 10^{10} units thereof to the recipient, a titer of the antibodies in the recipient is above 1:50,000, as is determined by ELISA.

[00140] According to another aspect of the present invention there is provided an agent for treating a plaque forming disease. The agent according to this aspect of the present invention comprising a display vehicle displaying a polypeptide, the polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being

capable of eliciting antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein.

[00141] According to still another aspect of the present invention there is provided a pharmaceutical composition for treating a plaque forming disease. The composition according to this aspect of the present invention comprising an effective amount of a display vehicle displaying a polypeptide, the polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable of eliciting an effective amount of antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein, and a pharmaceutically acceptable carrier.

[00142] According to yet another aspect of the present invention there is provided a method of preparing a display vehicle for treating a plaque forming disease. The method according to this aspect of the present invention is effected by genetically modifying a genome of a display vehicle by inserting therein a polynucleotide sequence encoding a polypeptide representing at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the at least one epitope being capable

of eliciting antibodies capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein, such that when the display vehicle propagates the polypeptide is displayed by the display vehicle.

[00143] Use of beta amyloid peptide antigens in conjunction with adjuvants to effect immunization has previously been difficult due to a combination of high toxicity and low titers which result. Using prior art methods as a starting point, immunization of a mouse with a 16 amino acids peptide of beta-amyloid conjugated to KLH (SEQ ID NO:9) was carried out. This immunization produced a low but measurable antibody titer against beta-amyloid.

[00144] While reducing one aspect of the present invention to practice, splenectomy of the immunized mouse facilitated preparation of IgM hybridoma 508 expressing scFvAb with specificity to beta-amyloid. RNA was subsequently extracted from this hybridoma and was employed for antibody cloning. IgM 508 hybridoma showed specific activity to A β in preventing its toxic affect on PC12 cells (Anavi, S. 1998). V_H and V_L sequences of IgM 508 were cloned separately and linked using a commercially available vector to form a single chain antibody with anti-beta amyloid specificity. This single chain antibody was subsequently expressed as a fusion protein in a

phage display library and clones with anti-beta amyloid activity were selected for propagation in *E. coli*.

[00145] Further reduction to practice was demonstrated by determining the apparent binding constants of the purified antibody-presenting phage to amyloid beta were measured by ELISA test, and half-maximal binding was obtained at an antibody concentration of 340 ng/ml, corresponding to 8×10^{-6} M. This result anticipates that the prepared single chain antibody will be effective under *in vivo* conditions. This phage was also able to disrupt already formed fibril structures confirming that the purified single chain antibody is biologically active, as suggested by the binding constant determination.

[00146] While reducing another aspect of the present invention to practice, it was uncovered that monoclonal antibodies raised against a peptide sequence of a prion protein were effective in disaggregating, or preventing the formation, of prion plaques.

[00147] A model for assessing PrP 106-126 toxicity was established by the present inventors and utilized to test the effectiveness of two immunoglobulin clones (designated mAb 3-11 (IgM) and mAb 2-40 (IgG1)) for neuroprotective and disaggregative capabilities.

[00148] As is further detailed in Examples 15-21, both mAb 3-11 and mAb 2-40 significantly reduced the dose dependent toxic effects of PrP 106-126 on PC-12 cells. Co-incubation of mAb 3-11 with PrP 106-126 prevented fibrillar aggregation, while administration of mAb 3-11 to already formed aggregates, resulted in disaggregation of 50% of the amyloid fibrils (Example 21).

[00149] Thus, according to an additional aspect of the present invention there is provided a method of treating a plaque forming disease. The method according to this aspect of the present invention is effected by displaying a polypeptide representing at least an immunological portion of an antibody being for binding at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the binding capable of disaggregating the aggregating protein and/or of preventing aggregation of the aggregating protein, and introducing the display vehicle into a body of a recipient so as to disaggregate the aggregating protein and/or prevent its aggregation.

[00150] According to a preferred embodiment of the present invention, and as is further described hereinbelow and exemplified hereinunder in the Examples section, introducing the display vehicle into the body of the recipient so as to disaggregate the aggregating protein and/or prevent the

aggregation of the aggregating protein is effected through an olfactory system of the recipient.

[00151] According to yet an additional aspect of the present invention there is provided an agent for treating a plaque forming disease. The agent according to this aspect of the present invention comprising a display vehicle displaying a polypeptide representing at least an immunological portion of an antibody which can bind at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the immunological portion of the antibody being capable of disaggregating said aggregating protein and/or of preventing aggregation of the aggregating protein.

[00152] According to still an additional aspect of the present invention there is provided a pharmaceutical composition for treating a plaque forming disease. The composition according to this aspect of the present invention comprising an effective amount of a display vehicle displaying a polypeptide representing at least an immunological portion of an antibody which can bind at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the immunological portion of the antibody being capable of disaggregating the aggregating protein, and a pharmaceutically acceptable carrier.

[00153] According to a further aspect of the present invention there is provided a method of preparing a display vehicle for treating a plaque forming disease. The method according to this aspect of the present invention is effected by genetically modifying a genome of a display vehicle by inserting therein a polynucleotide sequence encoding at least an immunological portion of an antibody capable of binding at least one epitope of an aggregating protein associated with plaque formation in the plaque forming disease, the immunological portion of the antibody being capable of disaggregating the aggregating protein.

[00154] For purposes of this specification and the accompanying claims the terms "patient", "subject" and "recipient" are used interchangeably. They include humans and other mammals which are the object of either prophylactic, experimental, or therapeutic treatment.

[00155] For purposes of this specification and the accompanying claims, the terms "beta amyloid peptide" is synonymous with " β -amyloid peptide", " β AP", " β A", and "A β ". All of these terms refer to a plaque forming peptide derived from amyloid precursor protein.

[00156] As used herein, "PrP protein", "PrP", "prion protein" and "prion" refer to polypeptides which are capable, under appropriate conditions, of inducing the formation of

aggregates responsible for plaque forming diseases. For example, normal cellular prion protein (PrP^{C}) is converted under such conditions into the corresponding scrapie isoform (PrP^{Sc}) which is responsible for plaque forming diseases such as, but not limited to, bovine spongiform encephalopathy (BSE), or mad cow disease, feline spongiform encephalopathy of cats, kuru, Creutzfeldt-Jakob Disease (CJD), Gerstmann-Straussler-Scheinker disease (GSS), and fatal familial insomnia (FFI).

[00157] As used herein in the specification and in the claims the term "disaggregating" refers to solubilization of aggregated proteins typically held together by non-covalent bonds.

[00158] For purposes of this specification and the accompanying claims the terms "comprising" refers to inclusion of one or more recited element but does not exclude other elements not specifically recited. For example, a polypeptide that comprises $\text{A}\beta$ peptide encompasses both an isolated $\text{A}\beta$ peptide and $\text{A}\beta$ peptide as a component of a larger polypeptide sequence. Similarly, an immunological portion of an antibody may be included as a part of a larger of the antibody, say the entire antibody.

[00159] As used herein in the specification and in the claims section that follows, the term "treating" includes substantially inhibiting, slowing or reversing the progression of a disease, substantially ameliorating clinical symptoms of a disease or substantially preventing the appearance of clinical symptoms of a disease.

[00160] As used herein in the specification and in the claims section that follows, the term "plaque-forming disease" refers to diseases characterized by formation of plaques by an aggregating protein (plaque forming peptide), such as, but not limited to, beta-amyloid, serum amyloid A, cystatin C, IgG kappa light chain or prion protein, in diseases such as, but not limited to, early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease, SAA amyloidosis, hereditary Icelandic syndrome, senility, multiple myeloma, and to prion diseases that are known to affect humans, such as for example, kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker disease (GSS), and fatal familial insomnia (FFI) and animals, such as, for example, scrapie and BSE.

[00161] Because amyloid plaques associated with the diseases described hereinabove are located within the brain, any proposed treatment modality must demonstrate an ability to cross the blood brain barrier (BBB) as well as an ability to

dissolve amyloid plaques. Normally, the average size of molecules capable of penetrating the BBB is approximately 2 kDa. Monoclonal antibodies are typically in the range 135-900 kDa. Therefore, future therapeutic use of antibodies in treating amyloid plaque diseases must rely on either reduction of their size concurrent with retention of activity, or on development of novel delivery strategies.

[00162] Small synthetic peptides consisting of antigen epitopes, such as the EFRH (SEQ ID NO:1) epitope of A β or the PrP 106-126 peptide (SEQ ID NO:25) described herein, are in general poor antigens and need to be coupled to a larger carrier. Even after coupling they may induce only a low affinity immune response. For example, injection of A β -KLH or A β -fibril leads to very slow immune response (Anavi, S., 1998) and many efforts have been made to circumvent low affinity response, with limited success.

[00163] Since the pathological effects of plaque-forming polypeptides are maintained only in the central nervous system (CNS), the capability of highly specific Abs in preventing such plaques *in vivo* is dependent on the permeability of the blood brain barrier (BBB). For example, in the progressive stage of AD, evidence shows alteration in the permeability of the BBB, which may lead to direct delivery of such antibody from the periphery to the CNS to disaggregate already formed

plaques and minimize further toxic effects (Schenk et al., 1999). Preferred embodiments of the present invention include direct delivery of monoclonal Abs presented on display vehicles to the brain across the blood brain barrier.

[00164] An increasing body of evidence shows that olfactory deficits and degenerative changes in the central olfactory pathways are affected early in the clinical course of AD. Moreover, the anatomic patterns involved in AD suggest that the olfactory pathway may be the initial stage in the development of AD.

[00165] Olfactory receptor neurons are bipolar cells that reside in the epithelial lining of the nasal cavity. Their axons traverse the cribriform plate and project to the first synapse of the olfactory pathway in the olfactory bulb of the brain. The axons of olfactory neurons from the nasal epithelium form bundles of 1000 myelinated fibers. This configuration makes them a highway by which viruses or other transported substances may gain access to the CNS across the BBB.

[00166] In the early stages of AD, the BBB may limit the entry of antibody circulating in the periphery to the CNS. In contrast, A β anti-aggregating antibodies displayed on a phage surface have the potential not only be delivered directly to the CNS by intranasal administration but also to prevent

olfactory permanent damage by β A in the patients. As previously shown, intranasal administration (Mathison et al., 1998; Chou et al., 1997 and Draghia et al., 1995) enables the direct entry of viruses and macromolecules into the CSF or CNS.

[00167] Use of olfactory receptor neurons as a point of delivery for an adenovirus vector to the brain is reported in the literature. This method reportedly causes expression of a reporter gene in the brain for 12 days without apparent toxicity (Draghia et al., 1995).

[00168] Thus, according to a preferred embodiment of the present invention, a vehicle displaying an immunological portion of an antibody capable of disaggregating, or preventing the formation of, a polypeptide aggregate associated with a plaque forming disease is delivered via this route to the brain.

[00169] As $A\beta$ is produced continuously by cells in peripheral tissues which cross the blood brain barrier (BBB) leading to localized toxic effects in specific neuronal populations, intranasal administration of such a vehicle may also prevent the progression of the disease by minimizing the amount of peripheral $A\beta$ available to form plaques.

[00170] The use of display vehicles such as filamentous phages as a drug delivery system to the CNS opens new horizons

for therapeutic approaches for Alzheimer's disease, as well as for other neurodegenerative diseases involving toxic extracellular aggregation of human peptides such as for example, prion generated diseases.

[00171] The display vehicle according to the present invention can be of any type including viral (e.g., bacteriophages, such as filamentous bacteriophages, fd, f88, f1, and M13 for example), bacterial and prion display vehicles. Thus, for example, the display vehicle can be a double stranded DNA virus, a single stranded DNA virus, an RNA virus (positive or negative strand), a bacteria and a polypeptide carrier. Preferably, the display vehicle is a filamentous bacteriophage such as fd, f88, f1, and M13. due to its linear structure, filamentous phage has high permeability to different kinds of membranes (Scott et al., 1990) and following the olfactory tract, it reaches the hippocampus area via the limbic system to target affected sites. The treatment of filamentous phage with chloroform changes the linear structure to a circular one, which prevents delivery of phage to the brain.

[00172] While the fd filamentous phage is used in the present examples and is the preferred phage sequence for use in the present invention, it should be understood that all filamentous phages are very similar and have the same gene

organization (Model et al, 1988). Thus, the principles of the present invention can be applied to any of the filamentous phages, such as M13, f1 and others.

[00173] According to a preferred embodiment of the present invention the display vehicle is capable of propagation in the recipient. Thus, for example, a bacteriophage display vehicle can be propagated in bacterial flora, such as *Escherichia coli* residing in the recipient's body. Alternatively, the display vehicle is an *in vivo* non-propagateable particle.

[00174] The phage or virus vehicle has promise as a targetable *in vivo* therapy approach. Although concerns about the potential infection of the natural intestinal flora (Delmastro et al., 1997; Willis et al., 1993; and Poul et al., 1999) have been expressed, UV inactivation of phage showed (Delmastro et al., 1997) that they are as immunogenic as their infective counterparts. Use of inactivated phage may preclude incorporation of phage encoded transgenes into the nucleus for subsequent expression in host cells (Larocca et al., 1998), an important practical consideration. Therefore, according to alternate preferred embodiments, the display vehicles employed in the present invention may be either replicating or non-replicating.

[00175] Phage or virus display involves the expression of cDNA clones as fusion proteins with phage or virus coat

proteins. If the cDNAs selected for expression encode antigens, the phage or virus may then be employed as an antigen presenting vehicle, which can optionally replicate within a recipient.

[00176] As described above, according to preferred embodiments of the present invention, antigens displayed by a phage or virus may be used directly for vaccination, without antigen purification. In this case, the bulk of the coat proteins serve to stimulate a general immune response because they are "non-self" with respect to the vaccinated subject. The antigen-coat protein fusion elicits a specific antibody against epitopes in the displayed cDNA gene product.

[00177] Antibody phage or virus display is accomplished, for example, by fusing the coding sequence of the antibody variable regions to a phage or virus coat protein. To this end, the variable (V) regions (V_H and V_L) mRNA isolated from antibody-producing cells is reverse-transcribed into cDNA, and heavy and light chains assembled randomly to encode single chain Fv (scFv). These cassettes are cloned directly into a suitable vector such as a phagemid vector for expression and display on the phage or virus surface. This linkage between antibody genotype and phenotype allows the enrichment of antigen specific phage or virus antibodies, using immobilized or labeled antigen. Phage or virus that display a relevant

antibody will be retained on a surface coated with antigen, while non-adherent phages or viruses will be washed away. Bound phages or viruses can be recovered from the surface, re-infected into suitable host cells and re-grown for further enrichment and, eventually for binding analysis.

[00178] The success of antibody phage or virus display hinges on the combination of this display and enrichment method. Phage or virus antibody genes can be sequenced, mutated and screened to improve antigen binding.

[00179] It is possible to rearrange the genes which code for the various regions of an antibody molecule such that its specificity and affinity for an antigen are altered. The antibody can be maintained on the surface of the phage or virus for further manipulation or be released as soluble scFv (~25 kDa) fragment.

[00180] Since its invention at the beginning of the 1990's, antibody phage display has revolutionized the generation of monoclonal antibodies and their engineering. This is because phage display allows antibodies to be made completely *in vitro*, bypassing the immune system and the immunization procedure, and allowing *in vitro* tailoring of the affinity and specificity of the antibody. It is therefore anticipated that the most efficient new vaccine development strategies will employ this technology.

[00181] Additional features can be added to the vector to ensure its safety and/or enhance its therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with the recombinant virus such as antibiotic sensitivity. Negative selection is therefore a means by which infection can be controlled because it provides inducible suicide through the addition of antibiotic. Such protection ensures that if, for example, mutations arise that produce altered forms of the viral vector or recombinant sequence, cellular transformation will not occur. Features that limit expression to particular cell types can also be included. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type.

[00182] Viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell.

[00183] Besides a method of treating a plaque-forming disease or disorder by applying to an olfactory system of the subject a viral display vehicle with an immunological

antigen/epitope-binding portion of an antibody displayed thereon, the present invention more generally comprehends a method of treating a neurological disease or disorder of the CNS using a phage display vehicle according to the invention for delivery of a therapeutic. These neurological diseases or disorders may be either plaque-forming diseases or non-plaque-forming neurological diseases or disorders of the CNS.

[00184] Moreover, the present invention also comprehends a method of diagnosing the presence/absence or extent of a neurological disease or disorder of the CNS using a viral display vehicle, such as the preferred filamentous bacteriophage display vehicle, for delivery of a diagnostic agent via an olfactory system of the subject to the affected site(s) in the brain. The delivered diagnostic agent is then detected by *in vivo* imaging, such as magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), or other commonly used *in vivo* imaging procedures. Protocols for NMR imaging and instrument procedures which include MRI, are found in texts such as Stark et al. (1992). Radiopharmaceutical Imaging Procedures are found in (Mettler et al., 1983; and Kim et al., 1987) and XRCM Imaging procedures are found in (Moss et al., 1992; and Sovak, 1984).

[00185] The diagnosis of Alzheimer's disease (AD) is currently only considered definitive upon neuropathological

examination of post-mortem brain tissue. Diagnostic tests are based on distinguishing disease-associated abnormalities involving the microtubule-associated protein tau and the beta-amyloid 42 peptide. Recent studies have shown that these proteins are also altered in the cerebrospinal fluid (CSF) of AD patients to the extent that the measure of these analytes has been proposed as useful for the *in vivo* diagnosis of AD. Trojanowski et al., (Alz. Dis. Rev. 1, 77 (1996) reviewed the data specifically relating to tau protein.

[00186] Recent postmortem studies indicate that increases in the total quantity of A β 1-42 in the hippocampus and in several cortical regions correlates highly significantly with both the initial appearance of clinical impairment and its subsequent progression. The latter work adds to a wealth of data supporting the cerebral accumulation of A β - in prefibrillar, diffusible forms as well as in fibrillar plaques - as the seminal pathogenic event in AD.

[00187] Although careful clinical and neuropsychological assessment remains the gold standard for determining whether a person probably has AD or is in a very early symptomatic phase (i.e., has minimal cognitive impairment), imaging the amount and regional distribution of amyloid could serve as a powerful adjunct, particularly in presymptomatic subjects. Sensitive psychometric screening to elicit subclinical signs of

cognitive dysfunction (analogous to a stress test in cardiological practice) may be coupled with functional *in vivo* imaging, such as magnetic resonance imaging of the brain. Particularly useful would be the direct imaging of A β deposits by SPECT or similar radioisotope methods, in order to solidify an impression of heightened risk (or early disease) emerging from the aforementioned tests. Taken together, these measures should result in a semiquantitative estimation of the probability of developing AD and help determine whether aggressive anti-amyloid therapy should be started.

[00188] Actually imaging the amounts of A β deposits in brain regions, such as hippocampus and temporal cortex, where AD-type dementia appears to begin could have an impact analogous to that of imaging clinically important atherosclerotic lesions in the coronary or carotid arteries. Such a delivery system will be able to target the plaques and to image A β deposits *in vivo*, and provide the most useful diagnostic and monitoring test for AD. Such "amyloid brain scans" would thus influence patient selection for trials, the monitoring of drug efficacy during and after those trials and the application of routine anti-amyloid treatment in the population. Moreover, the new brain delivery system according to the present invention, based on filamentous phages carrying anti-

aggregating antibodies directed against A β , can be used for disaggregation of the targeted amyloid plaques.

[00189] In addition to Alzheimer's disease and other plaque-forming diseases or disorders, non-plaque forming neurological diseases or disorders which can be treated and/or diagnosed using the phage display system for delivery of a therapeutic molecule or a diagnostic agent includes, but is not limited to, Parkinsonism (i.e., Parkinson's disease), Huntington's chorea, tardive dyskinesia, hyperkinesia, Tourette's syndrome, multi-infarct dementia, HIV dementia, dementia with or without Lewy bodies, attention deficit disorder, schizophrenia, epilepsy, occurrence of neuronal cell death such as from stroke or head trauma, global and focal ischemic and hemorrhagic stroke, Korsakoff's disease, cerebral palsy, migraines, CNS vasculitis, multiple sclerosis, narcolepsy, Down's syndrome, viral infections of the brain, brain tumors, Charcot-Marie-Tooth disease, neuropathies resulting from Lyme disease, adrenoleukodystrophy, mitochondrial myopathies, depression, anxiety, dyslexia, spino cerebellar degenerations, post encephalitic disorders, postapoplectic disorders, disorders attributed to CNS dysfunction, drug induced CNS disorders, neuropsychiatric disorders, mental illness, behavioral disorders, cognitive and cognitive affective disorders, and lysosomal storage diseases which cause

neurodegeneration and are manifested by enzyme deficiencies such as those listed in U.S. Patent 6,005,004 and in Neufeld, (1991).

[00190] General filamentous phage display delivery of any therapeutic molecule or diagnostic/imaging agent can be achieved according to one embodiment of the present invention by non-specific peptide inserts having either a site for biotinylation or an epitope which can bind to avidin or streptavidin. Any biotinylated therapeutic molecule or diagnostic agent can be captured by taking advantage of the avidity of a biotin/avidin-type affinity system. The binding constant of avidin-biotin is very high on the order of approximately 10^{15} M^{-1} .

[00191] Examples of the site (tag) inserted in the phage display for *in vivo* biotinylation in *Escherichia coli* is the 13 amino acid residue Bio tag (SEQ ID NO:30; Schatz, 1993; and Tucker et al., 1996) and the 15 amino acid residue BIOTIN AVITAG (SEQ ID NO:31; Avidity, Denver, CO or at www.avidity.com; U.S. Patents 5,932,433; 5,574,239; and 5,723,584). The Bio tag or the BIOTIN AVITAG displayed on a filamentous bacteriophage can be biotinylated *in vivo* in a specific *E. coli* strain (www.avidity.com) expressing biotin protein ligase or *in vitro* with biotin protein ligase enzyme (www.avidity.com) and biotin. As each molecule of avidin or

streptavidin has four high affinity binding sites for biotin, an avidin or streptavidin molecule can be used to bind a biotinylated therapeutic molecule or diagnostic agent to the biotinylated tag displayed on the surface of the filamentous phage used in the phage display delivery system according to the present invention. Examples of avidin-biotin binding are described in Bayer et al., (1980).

[00192] A non-limiting example of an epitope inserted for phage display which can bind to streptavidin is the 9 amino acid residue Strep tag (SEQ ID NO:32; Schmidt et al., 1993; Kleymann et al., 1995; and Tucker et al., 1999). This Strep tag binds specifically to streptavidin. Streptavidin can be used to bind a biotinylated therapeutic molecule or diagnostic agent to the Strep tag displayed on the surface of the filamentous phage used in the phage display delivery system according to the present invention.

[00193] As used herein, the term "biotin" or "biotinylated" is intended to encompass biotin, biocytin and other biotin analogs such as biotin amido caproate N-hydroxysuccinimide ester, biotin 4-amidobenzoic acid, biotinamide caproyl hydrazide and other biotin derivatives and conjugates. Other derivatives include biotin-dextran, biotin-disulfide-N-hydroxysuccinimide ester, biotin-6 amido quinoline, biotin hydrazide, d-biotin-N hydroxysuccinimide ester, biotin

maleimide, d-biotin p-nitrophenyl ester, biotinylated nucleotides and biotinylated amino acids such as N-epsilon-biotinyl-L-lysine.

[00194] It will be appreciated by those of skill in the art that "avidin" or "streptavidin" for binding to biotin encompasses avidin, streptavidin, deglycosylated avidin or streptavidin, recombinant or chemically synthesized avidin or streptavidin variants with amino acid substitutions or derivatives with chemical substitutions, as well as fragments, as long as such "avidin" or "streptavidin" will still accommodate biotin binding. One avidin derivative, EXTRAVIDIN can be obtained in various functionally derivatized or conjugated forms from Sigma Chemical Company (St. Louis, MO). Another example of an avidin derivative is NEUTRALITE AVIDIN (Belovo Chemicals, Bastogne, Belgium), a deglycosylated form of avidin, which was obtained enzymatically, exhibits a neutral pI, and bears free lysine groups for further derivatization.

[00195] Therapeutic molecules for delivery using the phage display vehicle according to the present invention include anti-neoplastic tumor agents, anti-microbial agents, anti-parasitic agents, adrenergic agents and catecholaminergic agents, anti-convulsants, nucleotide analogues, anti-trauma agents, enzymes and proteins used to prevent or treat

neurological diseases or disorders, etc. These therapeutic molecules include chemotherapeutic agents or immune activating drugs such as tissue plasminogen activator, adriamycin, vincristine, urokinase, streptokinase, methotrexate, cytarabine, thioguanine, doxorubicin, 5-fluorouracil, cisplatin, etoposide, ifosfamide, asparaginase, deoxycoformycin, hexamethyl melamine Ara-C, melphalan, and other folate analogs, daunomycin, doxorubicin, mitomycins, bleomycins, mitoxantrone, dactinomycin, etc. as well as toxins such as ricin, abrin, diphtheria toxin, Pseudomonas exotoxin A, ribosomal inactivating proteins, mycotoxins, etc. The chemotherapeutic agents are preferably those that do not cross the blood-brain barrier and is characterized by poor bioavailability.

[00196] Verotoxin or a shiga-like toxin (SLT), which refers to a group of toxins produced by enterohemorrhagic *E. coli* that resemble the *Shigella*-produced shiga toxins as is commonly understood in the art (U.S. Patents 5,968,894 and 6,121,242) is particularly preferred for brain tumors, such as astrocytoma. Delivery of a specific toxin, like verotoxin, to brain tumors induces apoptosis of tumor cells, and the complete, rapid, long-term elimination of human astrocytoma xenografts in nude mice (Arab et al., 1999).

[00197] The therapeutic molecule can also include lysosomal enzymes (for treating lysosomal storage diseases) such as ceramidase, glucocerebrosidase, beta-galactosidase, beta-hexosaminidase A, beta-hexosaminidase A & B, galactosylceramidase, arylsulfatase A, sphingomyelinase, alpha-galactosidase B, aspartylglycosaminidase, alpha-L-fucosidase, iduronate sulfatase, alpha-L-iduronidase, glcNAc-6-sulfatase, beta-glucuronidase, their recombinant analogs and their derivatives. Also included are serum proteins namely immunoglobulins, interleukins, interferons, hormones, such as insulin, parathyroid hormone, pigmentary hormone, thyroid-stimulating hormones, tissue plasminogen activator, nerve growth factors, peptidases or proteases, nucleic acids and derivatives thereof, nucleotides, oligonucleotides, antisense oligonucleotide analogs, genes, transfected cells, biological vectors, cloning vectors and expression vectors. Neurotoxins or their non-toxic peptide fragments are additionally included.

[00198] When it is desirable to have specificity in delivery of the therapeutic molecule or diagnostic agent to specific areas, tissues or cells of the brain, a molecule that acts as a specific targeting agent is preferably also delivered with the phage display vehicle according to the present invention. It is even more preferable and advantageous when the

therapeutic molecule is also the targeting agent, such as in the case of an antibody or a polypeptide having an antigen-binding portion capable of binding to amyloid β .

[00199] When targeting is required or preferred, any site-specific ligand for a molecular epitope or receptor to be targeted may be used. It is already understood that antibodies, antigen-binding fragments thereof, or a polypeptide having an immunological portion of an antibody can be a site-specific ligand for a molecular epitope or receptor. Other ligands as targeting agents include viruses, chemotherapeutic agents, receptor agonists and antagonists, lectin, albumin, peptides, hormones, amino sugars, lipids, fatty acids, and nucleic acids. Specific examples include neurotoxin fragments compatible with a receptor on a specific cell surface, (e.g., tetanus toxin fragment-C (TTC) for cerebral cortical neuronal cells and a nontoxic alpha-bungarotoxin (ABT) fragment for nicotinic acetylcholine receptor of hippocampal neurons) or nerve growth factor (NGF) for cholinergic neurons in general and the neurons of the basal ganglia of Meynart, in particular. U.S. Patent 6,033,644 also discloses biomodulators which can be considered to be targeting molecules that condition aberrant tissue to enhance uptake of therapeutic molecules or otherwise non-specific diagnostic imaging agents.

[00200] For targeting agents that are peptidic, these peptidic targeting agents can be displayed on the surface of filamentous bacteriophage in the same way that peptides or ScFv are displayed. Otherwise, a targeting agent which is non-peptidic can be linked to the surface of the filamentous phage by being biotinylated and making use of the Bio, BIOTIN AVITAG, or Strep tags and the avidin/streptavidin system described above. Biotinylation is well known and conventional in the art and the biotinylation of many different types of molecules has been reported in the literature and is within the skill of those in the art. Furthermore, it should be appreciated that it is possible to link a targeting agent or a therapeutic molecule to the surface of a filamentous phage by biofunctional linkers. Chemical linkage is understood herein as being by covalent bonds, conjugation or the formation of a complex.

[00201] The direct brain delivery of antibodies overcomes crossing the BBB by using olfactory neurons as transporters to the brain. In the olfactory epithelium, the dendrites of the primary olfactory neurons are in contact with the nasal lumen, and via the axons, these neurons are also connected to the olfactory bulbs of the brain. Phages that come into contact with the olfactory epithelium can be taken up in the primary

olfactory neurons and be transported to the olfactory bulbs, and even further into other areas of the brain.

[00202] Filamentous phages displaying specific antibodies for targeting the plaques of AD or prions can be labeled with contrast agents such as paramagnetic metals, e.g., gadolinium, technetium 99, etc., using chelating agents such as the diamide dimercaptide ligand system. Gadolinium (III) diethylenetriamine pentaacetic acid complex is the metal ion complex used for MRI in the diagnosis of cerebral tumors, CNS diseases, hepatic tumors, pituitary adenomas, multiple sclerosis and BBB impairment. A histidine tail (SEQ ID NO:33) can be used to immobilized heavy metals such as manganese, a metal with paramagnetic properties that is useful for MRI.

[00203] A very wide range of diagnostic agents detectable by diagnostic imaging is known in the art and the diagnostic agent will be selected according to the *in vivo* imaging technique to be used. For instance, in SPECT, radioactive ¹²⁵I is preferably used. Examples of suitable diagnostic agents to serve as reporter molecules/contrast agents are widely known in the diagnostic imaging literature as are chelating groups for use with metals. U.S. Patent 6,051,207 and the Description of the Related Art section of this application disclose non-limiting examples of diagnostic agents and chelating groups.

[00204] As an example of using antibodies to detect a disease state other than AD, a labeled antibody against ubiquitin or labeled polypeptide having an immunological antigen-binding portion of an antibody against ubiquitin can be used to target and detect Lewy bodies associated with dementia by *in vivo* imaging.

[00205] With regard to detecting the beta-amyloid protein of AD, biotinylated Chrysamine-G (CG), a carboxylic analog of Congo red, which is a histologic dye that stains amyloid, can be labeled and linked to the surface of filamentous bacteriophage via the Bio, Strep, or BIOTIN AVITAG system with avidin/streptavidin. The delivery of a phage display vehicle with labeled Chrysamine-G displayed on the phage surface provides a means of detecting beta-amyloid protein by *in vivo* imaging.

[00206] When the targeting agent is not labeled with a diagnostic agent or is not the same as the therapeutic molecule and must be delivered in addition to a diagnostic agent or a therapeutic molecule using the phage display vehicle, then those of skill in the art of filamentous bacteriophage would readily recognize that, for example, the diagnostic agent can be presented on another phage (i.e., f88). This phage can then be used as a helper phage for a phage displaying a scFv as a targeting agent. In this way,

the helper phage provides phage packaging functions and the resultant packaged phage displays both the specific scFv and the diagnostic agent on its surface.

[00207] As used herein in the specification and in the claims section that follows, the term polypeptide refers to a stretch of amino acids covalently linked there amongst via peptide bonds. Different polypeptides have different functionalities according to the present invention. While according to one aspect a polypeptide is derived from an immunogen designed to induce an active immune response in a recipient, according to another aspect of the invention, a polypeptide is derived from an antibody which results following the elicitation of an active immune response, in, for example, an animal, and which can serve to induce a passive immune response in the recipient. In both cases, however, the polypeptide is encoded by a polynucleotide according to any possible codon usage.

[00208] As used herein the phrase "immune response" or its equivalent "immunological response" refers to the development of a beneficial humoral (antibody mediated) and/or a cellular (mediated by antigen-specific T cells or their secretion products) response directed against an aggregating protein (plaque forming peptide) in a recipient patient. Such a response can be an active response induced by administration

of immunogen or a passive response induced by administration of antibody or primed T-cells. A cellular immune response is elicited by the presentation of polypeptide epitopes in association with Class I or Class II MHC molecules, to activate antigen-specific CD4⁺ T helper cells and/or CD8⁺ cytotoxic T cells. The response may also involve activation of monocytes, macrophages, NK cells, basophils, dendritic cells, astrocytes, microglia cells, eosinophils or other components of innate immunity.

[00209] As used herein "active immunity" refers to any immunity conferred upon a subject by administration of an antigen.

[00210] As used herein "passive immunity" refers to any immunity conferred upon a subject without administration of an antigen. "Passive immunity" therefore includes, but is not limited to, administration of a replicating display vehicle which includes an immunological portion of an antibody presented on its surface to a recipient. Although replication of such a vehicle is active, the immune response is passive from the standpoint of the recipient.

[00211] For purposes of this specification and the accompanying claims the terms "epitope" and "antigenic determinant" are used interchangeably to refer to a site on an antigen to which B and/or T cells respond. B-cell epitopes

can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66, Glenn E. Morris, Ed. (1996). Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen. T-cells recognize continuous epitopes of about nine amino acids for CD8 cells or about 13-15 amino acids for CD4 cells. T cells that recognize the epitope can be identified by *in vitro* assays that measure antigen-dependent proliferation, as determined by ^3H -thymidine incorporation by primed T cells in response to an epitope (Burke *et al.*, 1994), by antigen-dependent killing (cytotoxic T lymphocyte assay, Tigges *et al.*) or by cytokine secretion.

[00212] The presence of a cell-mediated immunological response can be determined by proliferation assays ($CD4^+$ T cells) or CTL (cytotoxic T lymphocyte) assays. The relative contributions of humoral and cellular responses to the protective or therapeutic effect of an immunogen can be distinguished by separately isolating IgG and T-cells from an immunized syngeneic animal and measuring protective or therapeutic effect in a second subject.

[00213] As used herein and in the claims, the terms "antibody" or "immunoglobulin" are used interchangeably and refer to any of several classes of structurally related proteins that function as part of the immune response of an animal or recipient, which proteins include IgG, IgD, IgE, IgA, IgM and related proteins.

[00214] Under normal physiological conditions antibodies are found in plasma and other body fluids and in the membrane of certain cells and are produced by lymphocytes of the type denoted B cells or their functional equivalent. Antibodies of the IgG class are made up of four polypeptide chains linked together by disulfide bonds. The four chains of intact IgG molecules are two identical heavy chains referred to as H-chains and two identical light chains referred to as L-chains.

[00215] In order to produce polyclonal antibodies, a host, such as a rabbit or goat, is immunized with the antigen or

antigen fragment, generally with an adjuvant and, if necessary, coupled to a carrier. Antibodies to the antigen are subsequently collected from the sera of the host. The polyclonal antibody can be affinity purified against the antigen rendering it monospecific. Previous experience has shown that standard production of polyclonal antibodies is not the method of choice for preparation of disaggregating antibodies for plaque forming peptides due to problems of poor titer and toxicity.

[00216] In order to produce monoclonal antibodies, hyperimmunization of an appropriate donor, generally a mouse, with the antigen is undertaken. Isolation of splenic antibody producing cells is then carried out. These cells are fused to a cell characterized by immortality, such as a myeloma cell, to provide a fused cell hybrid (hybridoma) which can be maintained in culture and which secretes the required monoclonal antibody. The cells are then be cultured, in bulk, and the monoclonal antibodies harvested from the culture media for use. By definition, monoclonal antibodies are specific to a single epitope. Monoclonal antibodies often have lower affinity constants than polyclonal antibodies raised against similar antigens for this reason.

[00217] Monoclonal antibodies may also be produced ex-vivo by use of primary cultures of splenic cells or cell lines

derived from spleen (Anavi, S., 1998, Locking the N-terminal of the Alzheimer β -amyloid peptide prevents the neurotoxicity in cell cultures, M. Sc. Thesis). In order to produce recombinant antibody (see generally Huston et al, 1991; Johnson and Bird, 1991; Mernaugh and Mernaugh, 1995), messenger RNAs from antibody producing B-lymphocytes of animals, or hybridoma are reverse-transcribed to obtain complementary DNAs (cDNAs). Antibody cDNA, which can be full length or partial length, is amplified and cloned into a phage or a plasmid. The cDNA can be a partial length of heavy and light chain cDNA, separated or connected by a linker. The antibody, or antibody fragment, is expressed using a suitable expression system to obtain recombinant antibody. Antibody cDNA can also be obtained by screening pertinent expression libraries.

[00218] The antibody can be bound to a solid support substrate or conjugated with a detectable moiety or be both bound and conjugated as is well known in the art. For a general discussion of conjugation of fluorescent or enzymatic moieties see Johnstone & Thorpe, *Immunochemistry in Practice*, Blackwell Scientific Publications, Oxford, 1982. The binding of antibodies to a solid support substrate is also well known in the art. See for a general discussion Harlow & Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory

Publications, New York, 1988 and Borrebaeck, Antibody Engineering - A Practical Guide, W.H. Freeman and Co., 1992.

[00219] As used herein and in the claims, the phrase "an immunological portion of an antibody" include an $F(ab')_2$ fragment of an antibody, an Fab fragment of an antibody, an Fv fragment of an antibody, a heavy chain of an antibody, a light chain of an antibody, an unassociated mixture of a heavy chain and a light chain of an antibody, a heterodimer consisting of a heavy chain and a light chain of an antibody, a catalytic domain of a heavy chain of an antibody, a catalytic domain of a light chain of an antibody, a variable fragment of a light chain of an antibody, a variable fragment of a heavy chain of an antibody, and a single chain variant of an antibody, which is also known as scFv. In addition, the term includes chimeric immunoglobulins which are the expression products of fused genes derived from different species, one of the species can be a human, in which case a chimeric immunoglobulin is said to be humanized. Typically, an immunological portion of an antibody competes with the intact antibody from which it was derived for specific binding to an antigen.

[00220] Optionally, an antibody or preferably an immunological portion of an antibody, can be chemically conjugated to, or expressed as, a fusion protein with other proteins. For purposes of this specification and the

accompanying claims, all such fused proteins are included in the definition of antibodies or an immunological portion of an antibody.

[00221] As used herein the terms "immunogenic agent" or "immunogen" or "antigen" are used interchangeably to describe a molecule capable of inducing an immunological response against itself on administration to a recipient, either alone, in conjunction with an adjuvant, or presented on a display vehicle.

[00222] As used herein the term "adjuvant" refers to a compound that, when administered in conjunction with an antigen, augments the immune response to the antigen, but when administered alone does not generate an immune response to the antigen. Adjuvants can augment an immune response by several mechanisms including lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages.

[00223] A pharmaceutical preparation according to the present invention includes, as an active ingredient, a display vehicle displaying at least one epitope of an aggregating protein associated with plaque formation in a plaque forming disease, the at least one epitope being capable of eliciting antibodies capable of disaggregating the aggregating protein. Alternatively, a pharmaceutical composition according to the present invention includes, as an active ingredient, a display

vehicle displaying at least an immunological portion of an antibody being for binding at least one epitope of an aggregating protein associated with plaque formation in said plaque forming disease, said immunological portion of said antibody being capable of disaggregating said aggregating protein.

[00224] The preparation according to the present invention can be administered to an organism *per se*, or in a pharmaceutical composition where it is mixed with suitable carriers or excipients.

[00225] It is also intended that a pharmaceutical composition according to the present invention can be used to treat not only plaque-forming diseases but other non-plaque forming neurological diseases or disorders of the CNS such as those mentioned in a preceding section of this specification. Such a pharmaceutical composition includes a pharmaceutically acceptable carrier and an effective amount of a viral display vehicle, which is preferably a filamentous bacteriophage, displaying a therapeutic molecule and capable of treating a neurological disease or disorder of the CNS.

[00226] Further comprehended by the present invention is a pharmaceutical composition for diagnosing the presence or extent of a neurological disease or disorder of the CNS. This pharmaceutical composition includes a pharmaceutically

acceptable carrier and an effective amount of a viral display vehicle which displays a diagnostic agent capable of being detected by *in vivo* imaging.

[00227] As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

[00228] Herein the term "active ingredient" refers to the preparation accountable for the biological effect.

[00229] Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

[00230] Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch,

cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

[00231] Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

[00232] Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, but the preferred route of administration is by the olfactory system of a subject.

[00233] Alternately, one may administer a preparation in a local rather than systemic manner, for example, via injection of the preparation directly into the brain of a patient.

[00234] Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

[00235] Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in

conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

[00236] For injection, the active ingredients of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[00237] For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in

particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[00238] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[00239] Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or

magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

[00240] For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[00241] For administration by nasal inhalation, the active ingredients for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[00242] The preparations described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be

presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[00243] Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

[00244] Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

[00245] The preparation of the present invention may also be formulated in rectal compositions such as suppositories or

retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

[00246] Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

[00247] Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[00248] For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from *in vitro* and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired circulating antibody concentration or titer. Such information can be used to more accurately determine useful doses in humans.

[00249] Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or

experimental animals. The data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975).

[00250] Dosage amount and interval may be adjusted individually to provide plasma or brain levels of antibodies which are sufficient to prevent aggregation or disaggregate existing aggregates (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Binding assays can be used to determine plasma concentrations.

[00251] Dosage intervals can also be determined using the MEC value. Preparations should be administered using a regimen, which maintains plasma levels above the MEC for 10-90 % of the time, preferable between 30-90 % and most preferably 50-90 %.

[00252] Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a

plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

[00253] The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

[00254] Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an

appropriate container, and labeled for treatment of an indicated condition, as if further detailed above.

[00255] The present invention also relates to a method of detecting both the pathogenic and non-pathogenic form of a prion protein in a biological sample.

[00256] Thus according to another aspect of the present invention there is provided a method of detecting a presence or an absence of a prion protein in a biological sample, the method comprising the steps of: (a) incubating an anti-prion antibody or an immunological portion thereof with the biological sample; and (b) determining a presence or an absence of antibody-antigen complexes, thereby determining the presence or the absence of the prion protein in the biological sample.

[00257] It will be appreciated that such complexes can be detected via any one of several methods known in the art, which methods can employ biochemical and/or optical detection schemes.

[00258] Thus, this aspect of the present invention provides a method of assaying or screening biological samples, such as body tissue or fluid suspected of including a prion protein either in a native non-disease conformation or a disease related conformation.

[00259] The detection method according to this aspect of the present invention, can also be utilized for rapid and cost effective screening of products such as pharmaceuticals (derived from natural sources), foods, cosmetics or any materials which might contain prions.

[00260] It will be appreciated that such a detection method can also be utilized in an assay for uncovering potential anti-prion drugs useful in prevention or disaggregation of prion aggregates.

[00261] Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

[00262] Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

[00263] Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and

recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, (Sambrook et al., 1989; Ausubel, R. M., 1994; Ausubel et al., 1989; Perbal, 1988; Watson et al.; and Birren et al. 1998; methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; Cellis, J. E., 1994; Coligan J. E., 1994; Stites et al., 1994; and Mishell and Shiigi 1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; (Gait, M. J., (1984); Hames, B. D., and Higgins S. J., 1985; Hames, B. D. and Higgins S. J., 1984; Freshney, R. I., 1986; Perbal, B., 1984; Marshak et al., 1996) all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

[00264] Reference is made to the following materials and methods, which were employed in experiments described in the following examples.

MATERIALS AND EXPERIMENTAL METHODS

[00265] The following materials and experimental methods were employed while reducing the present invention to practice as is further demonstrated in the Examples that follow:

General recombinant DNA and phage techniques:

[00266] Standard recombinant DNA techniques were performed essentially as described (Sambrook et al., 1989). General protocols for antibody-phage display technology are from the Pharmacia Biotech (Uppsala, Sweden) Recombinant Phage Antibody System (RPAS).

Construction of 508 scFv on the phage display:

[00267] The 508 IgM hybridoma used as the source for antibody variable-region sequences was generated from splenocytes of a mouse that had been immunized with a peptide corresponding to the 16 amino terminal residues of β AP conjugated to keyhole limpet hemocyanin, used as a carrier. mRNA extraction, first strand cDNA synthesis, PCR amplification of variable heavy (V_H) and variable light (V_L)

sequences, and assembly of scFv cassettes, were done according to protocols essentially as described (Pharmacia Biotech RPAS manual). Assembled 508 scFv DNA was digested with *Sfi*I and *Not*I, and 100 ng were ligated with 150 ng of vector DNA prepared by digestion of phagemid pCC-Gal6(Fv) (Berdichevsky Y et al., 1999) with *Sfi*I and *Not*I. This phage-display system is designed to express the scFv in frame fusion protein with cellulose binding domain (CBD) derived from *Clostridium thermocellum* (Morag E et al., 1995). Ligated DNA was introduced into XL-1 Blue cells (Stratagene, La Jolla, CA) by transformation and transformants were plated onto 2 X YT Agar plates containing 100 µg/ml ampicillin and 1 % glucose for overnight growth at 37 °C.

Selection of β -amyloid binding scFv-CBD fusion proteins:

[00268] Individual clones were picked and grown, each in 5 ml 2 X YT, 1 % glucose, 100 µg/ml Ampicillin overnight at 30°C. IPTG was added at 1 mM for a 3 hr induction period. Soluble scFv-CBD fusion proteins were isolated from each clone by sonication of induced cell pellets. In order to identify functional soluble 508(Fv) from non-functional ones, 250 ng/well β -amyloid peptide were covalently bound to epoxy-coated microtiter plates for 16 hr at 4°C (Solomon, B., et al, 1996). The plates were washed with PBS/0.05 % - Tween 20

(PBST), and blocked with a mixture of 3 % bovine serum albumin and milk powder in PBS for 16 hr at 4°C. The plates were then washed and incubated with the soluble scFv-CBD recovered from the clones for 1 hr at 37°C. The bound antibody was detected with a rabbit anti CBD antiserum followed by HRP-conjugated goat anti rabbit antibodies. Plates were developed with the peroxidase chromogenic substrate ABTS and the signal was recorded with an ELISA microtiter plate reader at 405 nm. Positive phage clones (pCC-508(Fv)) were propagated and their DNA was sequenced using an automated model 373A DNA sequencer (Applied Biosystems, USA).

Production of 508(Fv)-CBD fusion proteins in E. coli:

[00269] For high level expression in *E. coli*, wild type (wt) and mutated 508(Fv) derivatives were cloned into the pFEKCA3 vector as described (Berdichevsky Y et al, 1999). This vector utilizes the strong T7 promoter for expression, where the T7 RNA polymerase gene is carried as a lac repressor controlled-IPTG inducible gene in *E. coli* BL21 (DE3) (Studier, F.W., et al., 1990). Upon IPTG induction, 508(Fv)-CBD proteins accumulated as insoluble inclusion bodies. They were recovered by the cellulose-assisted refolding method as previously described (Berdichevsky Y et al, 1999). SDS polyacrylamide gel electrophoresis (SDS/PAGE) was used to separate proteins

according to their molecular weight under denaturing conditions (Laemmli, 1970).

Stability assay of the purified 508(Fv)-CBD protein:

[00270] The activity of purified 508(Fv)-CBD protein was checked before and after storage at 4 °C for 7 days. 250 ng/well β -amyloid peptide was covalently bound to epoxy-coated wells of microtiter plates for 16 hr at 4 °C (Solomon B. et al., 1997). Wells were blocked with a mixture of 3 % bovine serum albumin and bovine hemoglobin in PBS for 2 hr at 37 °C, then washed and incubated with the 508(Fv)-CBD protein (0.5 μ g/ml or as otherwise specified) for 1 hr at 37 °C. Bound antibody fragments were detected by incubation with HRP-conjugated rabbit anti-mouse antibodies (BioMakor, Rehovot, Israel), diluted 1:5,000 and rabbit anti CBD diluted 1:10,000 in PBST for 1 hr at 37 °C. The bound antibody fragments were monitored as described above.

Construction of a phage library for the isolation the 508(Fv) β AP binding mutants:

[00271] Splicing overlap extension (SOE) PCR technique (Lefenbrve, B., et al., 1995) was used to replace VL cysteine codon 96 of 508(Fv) with other codons. pCC-508(Fv) DNA was

used as template. In a first step, the template DNA was amplified with the following primers:

[00272] The antisense primer 508-mut-FOR: 5'-CCCCCTCCGAAC GTSNATGGGTAACTcgatcgCTGATGGCAGTA-3' (SEQ ID NO:10) inserts a *PvuI* restriction site (underlined), where S represents nucleotides C or G and N represents A, C, T or G. This primer was used for the replacement of cysteine codon 96 with phenylalanine (F), leucine (L), serine (S), tyrosine (Y) or tryptophan codons. The primer *SfiI* 5'BACK: 5'-ATCTATGCggcccagccggccATG-3' (SEQ ID NO:11) inserts an *SfiI* site at the 5' end of the scFv. The resulting PCR product (*SfiI*-508mut) corresponds to the 5' half of 508(Fv)-CBD. In the second PCR step, the complete 508(Fv)-CBD was re-assembled by amplifying pCC-508(Fv) DNA with the *SfiI*-508mut PCR product from step 1 serving as the 5' end primer and CBD(BX): 5'-GTGGTGCTGAGTggatccta TACTACACTGCCACCGGG-3' (SEQ ID NO:12) as the 3' end primer. The final PCR product (*SfiI*-508mut-BX) is a complete 508(Fv)-CBD cassette with replacements at V_L codon 96 and an engineered *PvuI* restriction site as a silent mutation for analysis. *SfiI*-508mut-BX DNA was digested with *SfiI*, *PvuI* and *NotI* and ligated in a three fragment ligation with *SfiI* and *NotI* linearized pCC-Gal6(Fv) DNA which is a phagemid vector used to display an anti *E. coli* β -galactosidase scFv (Berdichevsky Y et al, 1999). The resulting ligated phagemid

DNA was introduced into *E. coli* XL-1-Blue cells by electroporation. Cultures of *E. coli* were used to produce displaying phage by rescue with M13K07 helper phage (Pharmacia Biotech, Uppsala, Sweden).

Affinity selection of β -amyloid binding 508mut-(Fv) displaying phage clones:

[00273] A sample containing rescued phage particles was subjected to one round of affinity selection (biopanning) and amplification. For the selection cycle, 0.5 μ g/ml biotinylated β -amyloid 1-16 amino acid peptide (β AP(1-16), acids 1-16 OF SEQ ID NO:3) in a total volume of 1 ml were used. The phages were pre-incubated with the biotinylated peptide for 2 hr at room temperature, and the reaction mixture was then layered on streptavidin-coated 30 mm polystyrene Petri dishes and incubated for 20 min at room temperature. Unbound phages were removed by extensive washing with PBST. The bound phages were eluted with 0.3 ml of 0.1 M HCl titrated to pH 2.2 with glycine. The eluate was neutralized with 80 μ l of 0.5 M Tris (HCl) pH 10, and used to infect *E. coli* XL-1-Blue cells. Individual bacterial colonies containing amplified phage particles were used as a template for colony PCR (Novagen Madison, USA) with primers *Sfi*I5'Back and CBD(BX). The PCR product of about the size of an intact scFv-

CBD fragment (about 1250 bp) was digested with the restriction enzyme *PvuI* and analyzed by agarose gel electrophoresis.

ScFv binding to biotinylated β AP(1-16):

[00274] Binding of scFv to β AP(1-16) was analyzed by ELISA. Coated plates with 50 μ l of 1 μ g/ml streptavidin in 0.1 M NaHCO_3 , pH 9.6, were washed three times with PBST and 50 μ l of 6 ng/ μ l biotinylated β AP(1-16) were then added to the wells and incubated for 30 min at 37 °C. Wells were blocked with a mixture of 3 % bovine serum albumin and bovine hemoglobin in PBS for 2 hr at 37 °C, then washed and incubated with the scFv (0.5 μ g/ml or as otherwise specified) for 1 hr at 37 °C. For inhibition experiments, peptides were pre-incubated with the antibody for 30 min at 37 °C before their addition to peptide coated wells. After washing, bound antibody fragments were detected as described above. β AP specific binding phage clones were propagated and their DNA was isolated and sequenced as described above.

Cell culture and β AP cytotoxicity assay:

[00275] Rat phenochromocytoma PC12 cells were cultured in DMEM supplemented with 5 % horse serum, 10 % fetal calf serum,

2 mM L-glutamine, and 100 units/ml penicillin/streptomycin and incubated at 37 °C under 5 % CO₂. For the neurotoxicity assay, cultured PC12 cells were seeded into a 96-well plate at a density of 10⁴ cells/100 µl/well in a serum-free medium supplemented with 2 M of insulin. The effect on the prevention of the neurotoxicity of βA was measured as follows: 0.12 mM β-amyloid that was incubated for a week at 37 °C for the generation of fibrils, and further incubated in the presence of 508F(Fv)-CBD or with the unrelated Gal6(Fv)-CBD at a molar ratio of βAP to scFv of 15:1 or 30:1 for 24 hr. The βA/antibody mixture was added to the wells containing PC12 cells. The plates were incubated at 37 °C for 2 days, after which cell viability was assessed by measuring cellular redox activity with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), as described (Sladowski, D. et al., 1993). The plates were incubated overnight at 37 °C. MTT reduction was determined colorimetrically using an ELISA microtiter plate reader set at 550 nm.

Aggregation of β-amyloid peptide measured by Thioflavin T (ThT) Fluorimetry:

[00276] Aggregation of β-amyloid peptide was measured by the Thioflavin T (ThT) binding assay, in which the fluorescence

intensity reflects the degree of β -amyloid fibril formation. ThT characteristically stains amyloid-like deposits (Levine, 1993) and exhibits enhanced fluorescence emission at 485 nm upon excitation at 435 nm when added to the suspension of aggregated β -sheet preparations. Aqueous solutions of 0.12 mM β AP in 0.1 M Tris (HCl) pH 7.1 were incubated at 37 °C for 1 week and further incubated in the presence of 508F(Fv)-CBD or with the unrelated Gal6(Fv)-CBD at a molar ratio of β AP to scFv of 15:1 or 30:1 for 24 hr.. The fluorescence was measured after addition of 1 ml of ThT (2 μ M in 50 mM Glycine, pH 9) with an LSB-50 Perkin Elmer Ltd., UK, spectrofluorimeter.

Preparations of phage delivery system:

[00277] 12 Balb/c-female mice were divided to four groups of 3 mice per group. One group was used as control. Following a single dose of 10^{11} phage particles (fd phage, taken from a 15-mer phage peptide library which was provided by George P. Smith, University of Missouri, Columbia, MO) administered intranasally, mice were sacrificed in intervals of 1, 14 and 28 days in each group and their brains were taken for further analysis.

Ability of phage carrying scFv to enter/remove β AP fragment from the brain:

[00278] ScFv-508F fusion to filamentous minor coat gpIII were used in order to investigate the ability of β AP anti-aggregating scFv to be carries by a fillamentous phage display system directly into the CNS.

[00279] This scFv was prepared from anti-aggregation hybridoma 508 as described above and preserved its specific binding activity. Nine Balb/c mice divided into three groups were treated as follows: Mice of a first group were treated with 0.2 ml of 10^{-3} M biotin β A(1-16) alone. Mice of a second group were treated with a mix of 10^{10} phage carrying 508F-scFv which were pre incubated with 0.2 ml of 10^{-3} M biotin β A(1-16) for 1 hr. Mice of a third group were used as control. Following a single dose applied intranasally, mice were sacrificed in each group in intervals of 1, 14 and 28 days and their brains were taken for further analysis.

Preparations of tissue sections:

[00280] Immediately following decapitation, brains were removed and cut into two halves along the mid-sagittal sinus. Randomly, one half-brain was fixed by immersion in 4 % paraformaldehyde solution in 0.1 M phosphate buffer for two hours in 4°C and then immersed for cold protection in 4.5 %

sucrose in 0.1 M PBS over night. The sections were then moved to 30 % sucrose for 2 hr in 4°C. Sections of coronal blocks containing the olfactory and hippocampus were put in OCT and cut with thick-nesses of 6 µm with a cryostat at - 20°C, and then taken up on glass slides. Slides were kept at -70°C. These slides were used for phage detection using an immunofluorescence technique.

[00281] The other mid-sagittal half-brain was used for preparing paraffin tissue section for histology. The section were fixed in 4 % paraformaldehyde for 2 hr, then transferred to 10 % formalin saline for 2 days in room temperature, followed by embedding in paraffin, and cut with thick-nesses of 4 µm on a microtom and then taken up on glass slides. The slides were kept at room temperature until used.

Detection of antigen in brain sections:

[00282] *Immunofluorescence:* Sections were blocked with 3 % bovine serum albumin in PBS for 30 min and then incubated with rabbit polyclonal serum anti-fd or anti-M13 (1:100) or Streptavidin coupled with PE (sigma) or CyTM 3 for 1 hr at 37 °C. Slides were then washed three times, 5 min each, in PBS, treated again with the blocking buffer for 5 minutes at room temperature, and then reacted with secondary CyTM 3 donkey anti-rabbit IgG (for phage detection) at 1:400 (sigma) or with

streptavidin coupled with CyTM 3 or PE, 1:50 dilution, for 1 hr at room temperature. Finally, the preparations were washed three times in PBS, observed using a fluorescence microscope at a final magnification of x 10, and recorded on film or using a Hamamatsu digital camera (C4742) and Metamorph (Universal Imaging; West Chester, PA) computer software.

Histology:

[00283] Six-micrometer sections were stained with hematoxylin and eosin. The stained sections were examined and photographed at a final magnification of x 40. Finally, the preparations were washed three times in PBS, observed on a microscope, and recorded on film.

Immunization with f3-YYEFRH:

[00284] Immunizations were performed with a genetically engineered fd phage carrying the peptide YYEFRH (SEQ ID NO:7) fused to its minor coat gpIII. Doses of 10^{10} phages per injection were used to immunize at 14-day intervals, through intraperitoneal injections. Mice were injected the phages with or without Freund's complete adjuvant (Difco) for the first injection and Freund's incomplete adjuvant (Difco) for the second injection. Following 7 days of each injections, the mice were bled and their serum were tested by ELISA for

antibody IgG reactivity for both phage coat proteins and for β A.

Epitope libraries:

[00285] The 15-mer phage-peptide library used in this study was provided by George P. Smith (University of Missouri, Columbia, MO). The library consists of about 1.9×10^9 phage particles and comprises a random peptide repertoire of 15 amino acid residues fused to coat glycoprotein VIII of the fd phage. Experiments with this library were carried out according to instructions of the provider (George P. Smith University of Missouri, Columbia, MO).

Biotinylation of antibodies:

[00286] For antibody biotinylation, 100 μ g of each antibody in 0.1 M NaHCO₃, pH 8.6, was incubated for 2 hr at room temperature with 5 μ g of biotinamidocaproate N-hydroxysuccinimide ester (Sigma, B 2643) from a stock solution of 1 mg/ml in dimethylformamide and dialyzed at 4 °C against phosphate-buffered saline (PBS; 0.14M NaCl/0.01 M phosphate buffer, pH 7.4) overnight.

Isolation of phage presenting epitopes from peptide library:

[00287] A library sample containing 10^9 infectious phage particles was subjected to three rounds of selection (biopanning) and amplification. For each selection cycle a biotinylated monoclonal antibody (1 $\mu\text{g}/\mu\text{l}$) in a total volume of 25 μl was used. The phage clones were pre incubated with the biotinylated antibody overnight at 4 °C, and the reaction mixtures were then layered in 1 ml of PBS containing 0.5 % Tween 20 on streptavidin-coated 30 mm polystyrene Petri dishes and incubated for 20 min at room temperature. Unbound phages were removed by extensive washing (10 times for 10 min each) in PBS/0.05 % Tween 20. The bound phages were eluted with 0.3 ml of 0.1 M HCl titrated to pH 2.2 with glycine. The eluate was neutralized and used to infect *E. coli* K91 cells. After three rounds of panning individual bacterial colonies containing amplified particles were grown on a microtiter plate and the selected phages were tested by ELISA for their ability to bind to the studied antibody, as described below.

Antibody binding to isolated phage:

[00288] Binding of antibodies to phage was analyzed by ELISA. Wells of microtiter plates (Maxisorb, Nunc) were coated with 50 μl (at dilution of 1:1000 in 0.1 M NaHCO_3 , pH 8.6) of rabbit anti-phage serum and incubated overnight at 4 °

C. The wells were blocked with a mixture of 3 % bovine serum albumin and hemoglobin at a ratio of 1:1 (in PBS) for 2 hr at 37°C. Coated plates were washed three times with PBS/0.05 % Tween 20, and 50 µl of enriched phage clones containing 10^{10} phage particles were added to the wells and incubated for 1 hr at 37 °C. After washing, the studied antibody was added (1 µg/ml or as otherwise specified) and allowed to bind to the coated plate overnight at 4 °C and the binding constant thereof was measured. Positive phage clones were propagated and their DNA were sequenced in the insert region at the Sequencing Unit of the Weizmann Institute of Science (Rehovot, Israel) by using Applied Biosystem Kit (United States, Applied Biosystem).

fd gpVIII phage display of $\beta A(1-16)$:

[00289] Coat glycoprotein VIII of filamentous phage is presented in approximately 2700 copies on the phage coat. The following oligonucleotides were prepared: sense - 5'-agctccGATGCTGAATTCGG TGATAGCGGCTACGAAGTGCATCATCAGAAacctgcag-3' (SEQ ID NO:13); and antisense - 5'-ggTTTCTGATGATGCACTTCGTAGC CGCTATCATGACGAAATTCAGCATCgg-3' (SEQ ID NO:14). These oligonucleotides were used to form a duplex (68-70 ° C, 10 minutes, followed by slow cool to room temperature) which

encodes for amino acids 1-16 of human β AP and contain a silent mutation of a specific restriction site (*EcoRI*) which is useful for further analysis. The duplex was phosphorylated and ligated into *HindIII*/*PstI* linearized f88-4 phagemid, which is a vector used to display fusion peptides on gpVIII of filamentous phage. The resulting ligated phagemid DNA was introduced into *E. coli* K91K cells by transformation and transformants were plated onto 2 x YT Agar plates containing 10 μ g/ml tetracycline and 1 % glucose for overnight growth at 37 °C. Individual bacterial colonies containing phage particles were used to inoculate 2YT medium containing 10 μ g/ml tetracycline for overnight growth at 37 °C for amplification. The DNA phagemid product obtained from each colony was analyzed by *EcoRI*. Positive clones were further amplified for antigen preparation.

Immunization with f88-EFRH:

[00290] Immunizations were performed with a genetically engineered fd phage carrying the peptide VHEPHEFRHVALNPV (SEQ ID NO:8) fused to its major coat glycoprotein VIII. Doses of 10^{10} phages per injection were used to immunize at 14-day intervals, through intraperitoneal injections. Mice were injected the phages with or without Freund's complete adjuvant

(Difco) for the first injection and Freund's incomplete adjuvant (Difco) for the second injection. Following 7 days of each injections, the mice were bled and their serum were tested by ELISA for antibody IgG reactivity for both phage coat proteins and for β A.

Inhibition of antibody binding to β -amyloid peptide:

[00291] The inhibition of antibody binding to β AP(1-16) by various small peptides was performed using 250 ng/well biotinylated β -amyloid peptides (1-16) bound covalently to ELISA plates as previously described. The plates were washed with PBS/0.05 % Tween 20 and blocked with a mixture of 3 % bovine serum albumin and hemoglobin, ratio 1:1 (in PBS) for 2 hr at 37 °C. The peptides were pre incubated with 1:3000 dilution of serum after third immunization with f88-EFRH for 30 min at 37 °C before their addition to β AP-coated wells and were left overnight at 4 °C therein. After washing, bound antibody was detected by incubation with HRP-conjugated rabbit anti-mouse immunoglobulin, as described above. The results were used to derive the IC₅₀, which is the half molar concentration of peptide that fully inhibits antibody binding. Peptides were synthesis by Applied Biosystems Synergy Model

430A in the Unit for Chemical Services of The Weizmann Institute of Science by solid-phase using Fmoc chemistry.

Cell culture and cytotoxicity assay:

[00292] Rat pheochromocytoma PC12 cells were cultured in DMEM supplemented with 5 % horse serum, 10 % fetal calf serum, 2 mM L-glutamine, and 100 units/ml penicillin/streptomycin and incubated at 37 °C under 5 % CO₂. For the neurotoxicity assay, cultured PC12 cells were seeded into a 96-well plate at a density of 10⁴ cells/100 µl/well in a serum-free medium supplemented with 2 M of insulin. The effect on the prevention of the neurotoxicity of βA was measured as follows: 0.12 mM β-amyloid that was incubated for a week at 37 °C for the generation of fibrils, and further incubated in the presence of serum of EFRH-phage immunized mice and serum of a non-relevant phage immunized mice at dilutions of 5:1 and 10:1 for 24 hr. The βA antibody mixture was added to the wells containing PC12 cells. The plates were incubated at 37 °C for 2 days, after which cell viability was assessed by measuring cellular redox activity with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), as described (Sladowski, D. et al., J. Immunol. Methods., 157:203-207,1993). The plates were incubated overnight at 37 °C. MTT reduction was

determined colorimetrically using an ELISA microtiter plate reader set at 550 nm.

Preparation of monoclonal antibodies against PrP 106-126

[00293] Mice immunized with synthetic peptide corresponding to the sequence of human PrP 106-126 (SEQ ID NO:25) (obtained from Chiron Technologies, Clayton Victoria, Australia) coupled to the larger carrier KLH were used for generating monoclonal antibodies following the fusion techniques of Kohler and Milstein (Kohler and Milstein 1975).

[00294] Hybridomas were tested for the production of peptide-specific antibodies by ELISA, as follows: Peptide PrP 106-126 was covalently attached to the epoxy groups of Eupergit-C coated 96 well plates (Solomon et al. 1992, 1993). The residual epoxy groups were blocked by incubating the plates with 3% skim milk (blocking solution). Undiluted hybridoma supernatants were applied for 1 hour at 37°C. Wells were excessively rinsed (as in each step of the procedure) and further incubated with horseradish peroxidase (HRP) labeled goat-anti-mouse antibodies specific for mouse IgG or IgM (diluted in blocking solution). After washing, antibody binding was visualized using ortho-phenyldiamine as substrate for HRP. Optical density was measured at 492nm. Selected monoclonal antibodies were scaled-up and purified according to

published procedures: IgG molecules on a protein A column (Harlow et al. 1988) and IgM on KaptiveM coloumn. Two mAbs, namely 2-40 and 3-11, were used for further studies. The mAb 3F4 was purchased from Senetek, Ca. USA.

Search for antibody's epitope location using phage display library

[00295] The antibodies 3-11 (IgM) and 2-40 (IgG) were biotinylated. The following libraries (provided by G.P. Smith) were searched to find the epitope of the antibodies studied, as previously described (Frenkel et al. 1998).

[00296] 1. fUSE5/15-mer library where foreign 15-mer are displayed on all 5 copies of pIII. 2. f88-4/6-mer library where foreign 6-mer are displayed on up to ~300 copies of pVIII (recombinant gene VIII encoding the peptide is inducible with IPTG)

[00297] The biopanning of the phages to find the antibodies' epitope was performed as previously described (Frenkel et al. 1998).

Competitive inhibition of antibodies bound to PrP by peptide NMKH

[00298] The ELISA competitive binding of the above antibodies to covalently bound PrP peptide was performed as

described above. The antibodies were preincubated with peptide NMKH at equimolar ratio before adding to the wells.

PrP 106-126 aggregation and immunocomplexation

[00299] *In vitro* aggregation of peptide 106-126 was induced by incubation of an aqueous solution of PrP 106-126 (10 mg/ml) for various time intervals at 37°C. The aggregated peptide was incubated either with monoclonal antibodies 2-40, 3-11 or 3F4 at conditions specified later.

Cytotoxicity assay of PrP 106-126 using PC12 cells

[00300] Rat pheochromocytoma PC12 cells were cultured in DMEM supplemented with 8% horse serum, 8% fetal calf serum, 2 mM L-glutamine and 100 units/ml penicillin/streptomycin and incubated at 37°C under 5% CO₂.

[00301] For the neurotoxicity assay, cultured PC12 cells were seeded on 96-well plates at a density of 2×10^4 cells/100 μ l/well in a serum-free medium supplemented with 2 μ M of insulin. Cells were treated for 3-5 days with 100 μ M PrP 106-126 preincubated 4-7 days at 37°C. The cell viability was assessed by the MTT assay which measures the activity of mitochondrial enzymes responsible for the conversion of the tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-

tetrazolium bromide (MTT) to a formazan product in viable cells (Hansen et al. 1989). MTT was added to the wells to a final concentration of 1 mg/ml and incubated with the cells for an additional 3 hours at 37°C. Cell lysis buffer (20% wt/vol SDS in a solution of 50% dimethylformamide, pH 4.7) was added, and the plate was incubated overnight at 37°C. MTT reduction was determined colorimetrically by measuring the optical density (OD) at 550 nm.

Prevention of PrP 106-126 neurotoxicity

[00302] The effect of mAbs on the inhibition of PrP 106-126 neurotoxicity was determined as follows: PrP 106-126 (10 mg/ml) was incubated for 7 days at 37°C to induce maximal aggregation of the peptide. Monoclonal antibodies 3-11, 2-40 and 3F4 were added for 1 hour to samples of 1mM of the already aggregated peptide. The antibody-peptide mixtures, as well as the aggregated peptide alone, were applied to the cells to a peptide final concentration of 100 μ M. Cell viability following a 3 day incubation at 37°C with the aforementioned reaction mixture, was assessed as described above. 100% viability was defined as the value of MTT assay for untreated cells.

Modulation of PrP conformation followed by Thioflavin T (ThT) fluorimetry assay

[00303] Increasing amounts of PrP 106-126 peptide (0-0.8 mg/ml) were incubated for 7 days at 37°C. Prion amyloid fibril formation was measured by the Thioflavine T (ThT) binding assay. The binding of ThT to amyloid fibrils of certain origins generates a specific fluorescent signal: a 114nm red shift in the excitation peak from 336nm of excitation spectrum of the free dye in solution to a new excitation peak at 450nm of the bound dye. Additionally the bound dye has an enhanced emission at 482nm (Naiki et al. 1989, LeVine 1993). The aggregation of the prion peptide was followed using samples of PrP 106-126 (0.3 mg/ml) in 0.1M Tris/HCl pH-7.1 incubated for 7 days at 37°C, either with or without mAbs 3-11, 2-40 and 3F4 at various dilutions. Disaggregation of already formed prion amyloid fibrils was measured using samples of PrP 106-126 that were incubated for 7 days at 37°C and then supplemented with the mAbs for an additional 24 hours. Fluorescence (emission at 482 nm after excitation at 435 nm) was measured after an addition of the samples to ThT (2µM in 50mM glycine, pH-9).

EXPERIMENTAL RESULTS

[00304] Examples 1-6 below relate to the production of a single chain version of the anti aggregating monoclonal antibody. Examples 7-8 below relate to delivery of peptide or antibody displaying phage to the brain. Examples 9-14 below relate to the production of high titers of anti-aggregating polyclonal antibodies by direct immunization with beta amyloid antigens displayed on a phage, and to characterization of these antibodies.

EXAMPLE 1

Generation of an IgM hybridoma 508

[00305] Immunization of a mouse with a 16 amino acid peptide of beta-amyloid (acids 1-16 of SEQ ID NO:3) conjugated to KLH (SEQ ID NO:9) was carried out as described hereinabove. Repetitious immunization eventually produced a low but measurable antibody titer against beta-amyloid. Subsequent splenectomy of the immunized mouse facilitated preparation of IgM hybridoma 508 expressing scFvAb with specificity to beta-amyloid. RNA was subsequently extracted from this hybridoma. The IgM 508 hybridoma showed specific activity to A β in preventing its toxic affects on PC12 cells (Anavi, S. 1998, M. Sc. thesis from the department of Molecular Microbiology and Biotechnology of the Tel-Aviv University, I).

*EXAMPLE 2**Cloning of the variable domains of the 508 IgM hybridoma as a scFv*

[00306] MAb 508 showed specific recognition of β -amyloid and prevented its toxic effects on PC12 cells (Anavi S., 1998). For cloning the 508 antibody as a scFv in a phage display vector, RNA was extracted from 10^8 508 hybridoma cells and was used as a source for antibody variable region coding sequences. RT-PCR was used to amplify the variable domains that were cloned into the phage display vector pCC-Gal6(Fv), as described in Materials and Methods. When hybridoma derived antibodies are cloned as scFvs, some of the clones may contain aberrant sequences that are not functional. Therefore, to identify phagemid clones carrying functional β -amyloid binders from the generated clones, 10 individual clones were picked at random and soluble scFv-CBD fusion protein was produced thereby. Figure 2 shows a physical map of plasmid pCC-508 which was used to express the 508-scFv. The CBD domain serves as an immunological detection of soluble scFv protein or as a novel approach in refolding of soluble scFv protein inclusion bodies of overexpressed protein (Berdichevsky Y et al, Protein Expr Purif., 17(2):249-59, 1999). The plasmid used for 508-scFv over expression is shown in Figure 3. The soluble scFv-CBD from the selected clones was incubated in wells of an

ELISA plate that has been coated with β -amyloid peptide. Of the analyzed clones, 50 % showed specific binding to β AP. Figures 1a-e demonstrate and illustrate the preparation of 508 scFv from IgM antibody. Figure 4 shows β AP binding by the scFv-CBD produced by a positive clone that was chosen for further analysis. PCR analysis was used to characterize its DNA insert. It was found that the positive clone (designated pCC-508(Fv)) contained an intact DNA insert (Figure 5). DNA sequencing of pCC-508(Fv) confirmed that the clone expresses an intact scFv fragment (see, Figures 11a-b and SEQ ID NOs:5 and 6, for nucleic and amino acid sequences, respectively, modified as further described below).

EXAMPLE 3

Site directed mutagenesis of 508-(Fv) antibody

[00307] The DNA sequencing analysis of pCC508-(Fv) revealed the unusual appearance of a cysteine residue at the position 96 of V_L CDR3 (Kabat, E. A. et al., 1991). The deduced amino acid sequence of V_L CDR3 is: H⁸⁹QRSSYPCT⁹⁷ (SEQ ID NO:15). The presence of an unpaired cysteine residue in a scFv may reduce its folding yield and also decrease its stability in solution and its storage half life. Therefore, 508(Fv) was subcloned into an expression vector and produced in *E. coli* as

described in Materials and Methods. Figure 6 summarizes the production process of 508(Fv)-CBD by the cellulose-assisted refolding method (Berdichevsky et al, 1999). Although 508(Fv)-CBD could be purified to near homogeneity (Figure 6 lane 7) by this method, it refolded relatively poorly and was unstable upon storage at 4 °C (Figure 7). It was assumed that substitution of the cysteine with a different residue may increase the production yield and stability of the soluble scFv without having an adverse affect on its affinity (Kirpriyanov, 1997).

[00308] For the replacement of the 508 V_L cysteine 96 codon SOE PCR was used, which enabled the replacement of Cys 96 with phenylalanine, leucine, serine, tyrosine or tryptophan codons. In addition, the PCR scheme employed permits the persistence of the cysteine residue at that position. 508(Fv) mutants were cloned into the pCC-Gal6(Fv) phage vector, resulting in the generation of a micro library (having 6 potential variant). The replacing residues chosen are generally acceptable at that CDR3 position, as they are found in various antibodies in the Kabat database (Kabat, 1991). However, different replacements may vary in their effect on β AP binding. To test which replacement maintains β AP binding, a single cycle of affinity selection was performed on the 508(Fv)-Mut micro phage library using biotinylated β AP(1-16)

as a capturing antigen. PCR amplification and restriction analysis was used to monitor the enrichment of library clones after the affinity selection cycle. When the 508Mut-(Fv)-CBD DNA is digested with *PvuI*, a typical restriction pattern is obtained upon agarose-gel electrophoresis and ethidium-bromide staining. The lower 750 and 500 bp fragments represent the 508Mut-(FV)-CBD DNA, while an intact 1250 bp fragment represent scFv-CBD from the pCC-Gal6(Fv) DNA which was used as a vector. It was found that before affinity selection the library was heavily contaminated with the pCC-Gal(Fv) vector DNA. This is evident from the fact that the DNA of 18/19 randomly picked library clones was not cleaved at the *PvuI* site engineered adjacent to 508 V_L position 96. Only one of the 19 analyzed clones showed the expected restriction pattern associated with a mutation (Figure 8a). However, after affinity selection, 5/11 randomly selected clones showed the expected restriction pattern (Figure 8b). This indicates an enrichment factor of about 10 fold, which demonstrates the ability of 508 scFv mutants to bind the β AP(1-16) epitope. The DNA sequences of the 5 mutants were determined and are shown in Table 1 below. Suitable replacements of 508 V_L cysteine 96 codon were found to be phenylalanine, serine or tyrosine.

TABLE 1

| Lane (Figure 8b) | Amino Acid Sequence | SEQ ID NO: |
|------------------|-------------------------------------|------------|
| 4 | $8^9\text{HQRSSYP-C}^{96}\text{-T}$ | 16 |
| 5 | $8^9\text{HQRSSYP-F}^{96}\text{-T}$ | 17 |
| 6 | $8^9\text{HQRSSYP-Y}^{96}\text{-T}$ | 18 |
| 8 | $8^9\text{HQRSSYP-F}^{96}\text{-T}$ | 19 |
| 11 | $8^9\text{HQRSSYP-S}^{96}\text{-T}$ | 20 |

EXAMPLE 4*The recognition of $\beta\text{AP}(1-16)$ by scFv 508 mutants.*

[00309] For further examination of mutated 508 scFv derivatives, the mutated genes were subcloned into an expression vector and overexpressed in *E. coli*, as described for the wild type protein above. The interactions of the various mutated 508-(Fv) proteins (Table 1) with $\beta\text{AP}(1-16)$ were tested in an ELISA assay. Figures 9a-b show that while the wild type 508-(Fv)-CBD binds at a half maximum binding (HMB) of 10^{-5} M, all the mutants showed improved binding to βAP : the HMB of C96S and C96Y is 5×10^{-6} M and the HMB of C96F is 10^{-7} M. For further examination the 508-scFv mutant that carries the C96F mutation (508F(Fv)) was chosen, which show the higher affinity and shelf stability (Figures 9a-b). The specificity of $\beta\text{AP}(1-16)$ binding by 508F(Fv) was tested in a

competitive ELISA. As is shown in Figure 10, binding of the purified 508F(Fv)-CBD to β AP was inhibited by soluble β AP(1-16) peptide serving as the competitor in the liquid phase in a dose-dependent manner. Inhibition of 50 % binding was obtained at about 1 μ M competitor. Binding was not affected by an irrelevant peptide (WVLD, SEQ ID NO:4).

EXAMPLE 5

Prevention of the β -amyloid neurotoxic effect by 508F(Fv).

[00310] In order to find out whether 508F(Fv) exhibits a protective effect similar to the parental IgM antibody in preventing β A mediated neurotoxicity toward cultured cells, an *in vitro* test was applied using rat phenochromocytoma PC12 as described (Solomon B. et al., 1997). Viability of the cells exposed to β A with or without antibody was measured. As shown in Figure 12, 508F(Fv) prevented the neurotoxicity of β A (90 % cell viability) at a molar ratio β AP:scFv of 15:1, while the unrelated scFv showed no effect. Purified CBD or the scFv alone had no affect on the cells.

EXAMPLE 6

Disaggregation of β -amyloid fibril by 508F(Fv)

[00311] To examine the effect of 508F(Fv) on disruption of the β A fibril (the toxic form of β AP) the ThT reagent that binds specifically to fibrillar structures (Levine, H. III, 1993) was used. The interference with the already formed β A fibril was measured at the same molar ratio of β AP:scFv as in the β A neurotoxic assay and was quantitated by ThT fluorimetry. Figure 13 shows that 508F(Fv) incubated with pre-formed β A fibrils disrupted the fibril structure indicating extensive deterioration of fibril morphology, as is evidenced by a substantial (62 %) decrease in ThT fluorescence.

EXAMPLE 7

Ability of filamentous phage to enter the CNS via olfactory track

[00312] Female Balb/c mice were treated with phage vector f88-EFRH via intranasal administration. The purpose of this experiment was to check the ability of filamentous phage to reach the hippocampous region via olfactory tract. Since the phage is not carrying any specific molecule for targeting neuron cells, it should be vanished without causing any harm after several day following the administration. In order to

investigate the appearance of phage in the olfactory bulb and the hippocampous region double labeling of antibodies was used as follows: Rabbit polyclonal antibody anti-filamentous phage and mouse monoclonal antibody against EFRH epitope fused to glycoprotein VIII of the phage surface. One day following a single intranasal administration of 10^{11} phages animals showed such phages in their olfactory bulb and hippocampous (Figures 14a-d). Seven days after the administration phages were detected in the olfactory bulb of only one mouse of the three tested, whereas no phages were revealed in the hippocampus. No evidence of phages was detectable 28 days following administration (Figures 15a-d). As shown in Figures 16a-d, no evidence of change in the neuron population of the brain of treated mice was evident.

EXAMPLE 8

Filamentous phage are suitable vehicle for carrying active antibody fragment to the CNS

[00313] To check whether a filamentous phage can carry an antibody to the CNS via the olfactory track and still preserve the activity against β -amyloid a filamentous phage displaying 508F was incubated with 10^{-3} M biotinlated β AP(1-16), in order to form antibody antigen immunocomplex. Balb/c mice were divided into two groups and were administrated intranasally

with two different antigens: 508F- β AP(1-16) immunocomplex and for comparison biotinlated β AP(1-16) alone. Following a single dose the mice were sacrificed and brain sections thereof prepared and reacted with streptavidin coupled to a fluorescent agent. Fluorescence was detected only in brain sections of mice that were administered with 508F- β AP(1-16), but not, or to a much lesser extent, in brain sections of mice that were administered with β AP(1-16) alone (Figures 17a-d). No histological findings characterized treated mice (Figures 18a-d).

[00314] It is therefore assumed that the phage act as an inert vehicle of antibody to the brain, carrying the β AP(1-16) molecule into the brain.

EXAMPLE 9

Raising anti-aggregating β AP antibody through immunization of mice with f3-EFRH phage

[00315] The anti-aggregating epitope within β AP (EFRH, SEQ ID NO:1) map to positions 3-6 of the amino acid sequence of β AP. In order to generate specific immune response against β AP, mice were immunized with genetically engineered fd phage carrying the peptide YYEFRH (SEQ ID NO:7) fused to its minor coat gpIII according to the immunization schedule shown in

Figure 19. Doses of 10^{10} phage particles per injection were used to immunize, at 14-day intervals, through intraperitoneal injection. Following 7 days of each injection, mice were bled and their sera tested by ELISA for IgG antibody reactivity against wild type phage (not bearing the peptide YYEFRH (SEQ ID NO:7) on its surface) and against β AP (Figures 20a,b).

This route of administration a very high response against β AP (1:750) following the third injection. Furthermore, it was found that injection through phage carrying epitope is long lasting (Figure 21), it is non-toxic and may be given without adjuvant. The phage vector is found to be an immunogenic tool to raise a high affinity immune response within 14 days from the first injection. The immune response against the peptide YYEFRH (SEQ ID NO:7) is low, compared to the immune response against the entire phage and could be explained by the low copy number of the fusion gpIII on the phage envelope. Therefore, for further analysis phages displaying the epitope through glycoprotein VIII were employed.

EXAMPLE 10

Isolation of f88-EFRH peptide-displayed phage by an anti-aggregating mAb

[00316] To identify a disaggregating EFRH peptide epitope a phage-epitope library was screened with biotinylated antibody.

After three cycles of panning and phage amplification, 90 individually isolated bacterial colonies were grown in microtiter plates and their phages were assayed for antibody binding. ELISA analysis revealed that of the phage-clones which were selected followed by three biopanning cycles, most (above 80%) bound specifically to anti-aggregating mAb, respectively. DNA from 6 positive clones was sequenced (Table 2). The sequence EFRH (SEQ ID NO:1) appeared in 4 clones, one additional clone had the sequence EPRH (SEQ ID NO:1), with only one residue replacement of proline with phenylalanine. In one additional clone, the inserted peptide bears the sequence of the three residues FRH (acids 2-4 of SEQ ID NO:1), lacking the glutamate residue.

TABLE 2

| Amino acid Sequence (name) | SEQ ID NO: | No. of Phages |
|-------------------------------|------------|---------------|
| VHEPHEFRHVALNPV (C3-II) | 8 | 2 |
| DTEFRHSSNNFSAVR (C7-II) | 21 | 1 |
| STEFRHQTTPLHPNS (C11-I) | 22 | 1 |
| KEPRHHIQHHERVIR (F8-II) | 23 | 1 |
| SAADFRHGSPPI SAF (D3-I) | 24 | 1 |

[00317] Binding of anti-aggregating mAb to the EFRH-bearing phage was concentration dependent; half-maximal binding was

obtained at an antibody concentration of 100 ng/ml, corresponding to 10^{-9} M (Figure 4) which resembles the level of binding of these antibodies to the whole peptide. One specific f88-EFRH phage (termed C3-II, table 2) showed higher level of avidity as is compared to the others (Figure 22). It may be due to higher level of EFRH epitope exposure on its surface. Binding tests of f88-EFRH or f3-EFRH with the same concentration of anti-aggregating antibody ($1\mu\text{g/ml}$) demonstrated a higher number of EFRH epitope copies per phage which may lead to higher serum titer via phage immune response (Figure 23).

EXAMPLE 11

Raising anti-aggregating β AP antibody through immunization of mice with f88-phage

[00318] In order to generate the same specific immune response against β AP, mice were immunized with genetically engineered fd phage carrying the peptide VHEPHEFRHVALNPV (SEQ ID NO:8) fused to its major coat gpVIII. This phage was selected from a 15-mer phage peptide library by an anti-aggregating β AP antibody and is presenting the mAb epitope (underlined) within β AP. This phage was used to immunize mice as described. Following 7 days of each injection with 10^{10} phage particles (without adjuvant) the mice were bled and

their sera tested by ELISA for IgG antibody reactivity against wild type phage and against β AP. The results are summarized in Figures 24a-b. All animals showed a measurable response of IgG antibody against the wild type phage, and titers increased with the second and the third injection. This route also gave the highest titer measurable responses against β AP (1:50,000) after the third injection (Figure 24b).

EXAMPLE 12

Inhibition of antibody serum binding to β -amyloid peptide

[00319] The interaction of mouse serum immunized by phage f88-EFRH with β AP was further assayed by competitive inhibition experiments. Figure 25 shows inhibition of mice serum antibody with synthesized peptides derived from β AP, each of which includes the sequence EFRH) such as: DAEFRH (positions 1-6, SEQ ID NO:3), DAEFRHD (positions 1-7, SEQ ID NO:3), DAEFRHDSG (positions 1-9, SEQ ID NO:3), and β AP itself, DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVG GVV (positions 1-40, SEQ ID NO:3).

[00320] Figure 25 shows that all of the synthetic peptides which bear the motif EFRH (SEQ ID NO:1) similarly inhibited binding of mouse serum antibody to the β AP with IC₅₀ values of about 5×10^{-6} M. These data indicate that the epitope of mouse

serum antibody within the β AP molecule is composed of four amino acid residues corresponding to positions 3-6 in the β AP which was found to act as a regulatory site controlling both the solubilization and the disaggregation process of the β A molecule.

EXAMPLE 13

Prevention of the β -amyloid neurotoxic effect by serum antibody raised against EFRH-phage

[00321] In order to find out whether serum of f88-EFRH immunized mice exhibits a protective effect similar to the parental IgG antibody in preventing β A mediated neurotoxicity toward cultured cells, the *in vitro* test using rat phenochromocytoma PC12 was applied as described (Solomon B. et 1997). Viability of the cells exposed to β A with or without antibody was measured. As shown in Figure 26, diluted serum of 1:5 prevented the neurotoxicity of β A (80 % cell viability), while the unrelated serum showed no effect.

EXAMPLE 14

Disaggregation of β -amyloid fibril by serum of EFRH immunized mice

[00322] To examine the effect of serum of f88-EFRH immunized mice on disruption of the β A fibril (the toxic form of β AP)

the ThT reagent that binds specifically to fibrillar structures (Levine, H. III, 1993) was used. β AP samples were incubated for a week at 37°C and then were exposed to different dilutions of mouse serum antibody. Fibril formation was quantitated by the ThT fluorometry binding assay. Figure 27 shows that mouse serum, at dilution of 1:5 and 1:20, disrupted the fibril structure of β A with extensive deterioration of fibril morphology, as indicated by a substantial 75 % (1:5 dilution) and 50 % (1:20 dilution) decrease in ThT fluorescence. The unrelated serum used as control (serum from non-immunized mouse), did not significantly inhibit fibril formation as is compared to the immunized serum. This result strongly emphasizes the ability of the EFRH epitope displayed by a filamentous phage vector to evoke an immune response resulting in anti-aggregation antibody.

EXAMPLE 15

[00323] The teachings of the present invention can also be applied to prion related diseases which are also characterized by plaque formation.

[00324] The possible involvement of the PrP protein in the pathogenesis of nerve cell degeneration and glial cell reaction led to the identification of a PrP sequences that

play a role in the amyloid formation. A fragment of PrP consisting of amino acids 106-126 was demonstrated to be toxic to rat hippocampal neurons (Forloni et al. 1993), to mouse cortical and cerebellar cells (Brown et al., 1994; 1997), and to be particularly highly fibrillogenic (Selvaggini et al. 1993). The formed fibrils were partially resistant to proteases digestion and exhibited properties of *in situ* amyloid (Selvaggini et al. 1993, Tagliavini et al. 1993). Synthetic peptides corresponding to this region of PrP exhibit conformational flexibility from α -helix to a β -sheet conformation (Selvaggini et al. 1993) which was similar to conformational changes from PrP^C to PrP^{Sc}. The conformational plasticity of this region is further emphasized by the findings that two distinct prion strains which exhibit different sites of proteolytic cleavage within this region (Bessen and Marsh, 1994; Telling et al., 1996).

[00325] As is further described below, the inventors of the present invention have generated mAbs specific to epitopes formed by amino acids 106-126 of the PrP protein. Such antibodies are usefull in studying plaque formation and morphology and as possible active agents for treating or preventing prion generated plaque diseases.

Preparation of monoclonal antibodies against PrP 106-126

[00326] Mice immunized with a synthetic peptide corresponding to the amino acid sequence of human PrP 106-126 coupled to the larger carrier KLH were used for generating monoclonal antibodies reactive against epitopes on this peptide. Sera derived from the immunized mice was subjected to ELISA and several positive clones composed mainly of immunoglobulin M (IgM) molecules and to a lesser extent IgG molecules were detected and isolated.

[00327] Two immunoglobulin clones that were isolated as described above and designated mAbs 3-11 (IgM) and mAb 2-40 (IgG1) were utilized in further studies.

*EXAMPLE 16**Search for epitope location using phage display library*

[00328] Phage display libraries displaying various peptide fragments of the human PrP 106-126 polypeptide were generated as described hereinabove in the method section. Clones reactive to mAbs 3-11 or mAb 2-40 (for each library) were not detected following 6 cycles of library biopanning (368 clones screened) raising the possibility that epitopes that are recognized by these antibodies are of a conformational nature.

*EXAMPLE 17**Competitive inhibition of antibodies bound to PrP by peptide NMKH*

[00329] The above described anti-PrP 106-126 antibodies were preincubated with a peptide of an amino acid sequence NMKH (SEQ ID NO:26) at equimolar ratio before reacting with the PrP 106-126 polypeptide in order to determine the ability of NMKH to compete with the human PrP 106-126 for antibody binding. As shown in Figure 28, this sequence is highly conserved between mice and human sequences (and others) with the exception of two different amino acids, M109 instead of L and M 112 instead of V. These two differences probably contribute to antigenic differences between the mouse and human sequences, since the antibodies which were derived from mice immunized with the human sequence corresponding to PrP 106-126 did not cross react with the mouse sequence corresponding to peptide PrP 106-126.

[00330] Competition (as analyzed by ELISA) between the NMKH and whole peptide was not detected, supporting the suggestion that the epitope may depend upon three dimensional conformation and not primary structure. In addition, co-reacting mAb 3F4 (recognizing the sequence MKHM) and mAb 3-11 with PrP 106-126 did not produce an additive response, suggesting that their epitopes may be adjacent or overlapping (Solomon and Balas, 1991).

*EXAMPLE 18**PrP aggregation and immunocomplex formation*

[00331] Incubation of PrP 106-126 at 37 °C at different concentrations led to a dose dependent fibrillar aggregation as measured by the Thioflavin T binding fluorescence assay (Figure 30). A PrP 106-126 concentration of 0.3 mg/ml was selected for further studies.

*EXAMPLE 19**Cytotoxicity assay of PrP 106-126 using PC12 cells*

[00332] Since the major target organ for PrP^{Sc} is the nervous system, *in vitro* neuronal model systems were used to analyze PrP toxicity. A clonal cell line which exhibits neuronal properties has been established from rat central nervous system tumors (Schubert et al. 1974). The rat pheochromocytoma PC12 cells - a neuron-like cloned tumor cell line (Greene and Tischler, 1976) - has been shown to enable *in vitro* replication of PrP^{Sc} (Rubenstein et al. 1984). As such, this cell line was utilized by the present study as a model for detecting molecules with the ability to prevent toxicity induced by PrP fibrils.

[00333] Using this model system, it was uncovered that PrP 106-126 is toxic to PC12 cells in a dose dependant manner, which toxicity is related to the conformational state of the

peptide and to the exposure time of the cells to aggregated peptide. Cell viability considerably decreased (as detected by MTT assay), when cells were incubated with PrP 106-126 for 5 days in comparison to a 3 day exposure. Furthermore, preincubation of PrP 106-126 at 37 °C prior to its addition to the cells increased its toxicity probably by increasing the amount of amyloid fibrils. Preincubation of the peptide for 7 days resulted in a significantly higher toxicity which was concentration dependent (Figure 29). Under the described conditions, cell viability was reduced to 10% at a concentration of 100µM of PrP 106-126 as was measured by MTT assay.

EXAMPLE 20

Prevention of PrP 106-126 neurotoxicity

[00334] The cytoprotective effect of mAb 3-11 and 2-40 is shown in Figure 31. Mabs 3-11 and 2-40 inhibited cell death which was induced by 100µM PrP 106-126. The viability of cells treated with a mixture of either antibody and the peptide was 85-89%, as compared to a 40% survival rate in cells treated with the peptide alone (The antibodies without the peptide had no affect on cell viability). The antibodies' protective effect was apparently related to the specific

epitope on the PrP molecule, since such protection was not demonstrated with mAb 3F4 (44% viability).

EXAMPLE 21

Anti-aggregating properties of monoclonal antibodies as measured by ThT assay

[00335] The amyloid fibril formation of PrP 106-126 was detected by a thioflavin-T (ThT) binding assay. Both mAbs 3-11 and 2-40 prevent PrP 106-126 fibrillar aggregation and disaggregate the aggregated form of this peptide. A significant decrease in amyloid fibril formation (about 80% prevention) was observed when PrP 106-126 was incubated at

[00336] 37 °C in the presence of mAbs 3-11 and 2-40. When these antibodies were added to already formed aggregates of PrP 106-126, more than 50% of the fibrils were disaggregated. In contrast, mAb 3F4 had lower efficacy both in inhibiting amyloid fibril formation and in inducing their disaggregation (Figure 32). Both the inhibition of amyloid fibril formation and the induction of disaggregation were dependent on the antibody's concentration and the epitope location (Figures 32-33).

[00337] The protective effect of mAbs 3-11 and 2-40 of the present invention and of mAb 3F4 was calculated as shown in Equation 1 below.

[00338]

$$\%Protective\ effect = 100 - \frac{Emission\ 482nm(Pr\ P106 - 126\ incubated\ with\ Ab)}{Emission\ 482nm(Pr\ P106 - 126\ incubated\ alone)} \times 100$$

(Equation 1)

[00339] Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

[00340] While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

[00341] All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and

text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by references.

[00342] Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

[00343] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the

teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

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WHAT IS CLAIMED IS:

1. A method of treating a neurological disease or disorder of the central nervous system (CNS), comprising: displaying a therapeutic molecule capable of treating the neurological disease or disorder of the CNS on a viral display vehicle; and

introducing the viral display vehicle into a subject in need thereof by applying an effective amount of the viral display vehicle displaying the therapeutic molecule to an olfactory system of the subject to treat the neurological disease or disorder.

2. The method of claim 1, wherein the neurological disease or disorder of the CNS is a plaque-forming disease or disorder.

3. The method of claim 2, wherein the plaque forming disease or disorder is selected from the group consisting of early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease, SAA amyloidosis, hereditary Icelandic syndrome, senility and multiple myeloma.

4. The method of claim 2, wherein the plaque forming disease or disorder is selected from the group consisting of scrapie, bovine spongiform encephalopathy (BSE), kuru, Creutzfeldt-Jakob Disease (CJD), Gerstmann-Streussler-Sheinker Disease (GSS) and fatal familial insomnia (FFI).

5. The method of claim 2, wherein the therapeutic molecule displayed on the viral display vehicle is a polypeptide comprising an immunological portion of an antibody that binds at least one epitope of an aggregating protein associated with plaque formation in the plaque-forming disease or disorder, the binding of the immunological portion of the antibody to the aggregating protein being capable of disaggregating the aggregating protein and/or of preventing or inhibiting aggregation of the aggregating protein.

6. The method of claim 5, wherein the aggregating protein is selected from the group consisting of beta-amyloid, serum amyloid A, cystatin C, IgG kappa light chain, and prion protein.

7. The method of claim 5, wherein the aggregating protein is prion protein and at least one epitope of the prion protein as the aggregating protein is defined by at least a portion of an amino sequence of SEQ ID NO:25.

8. The method of claim 1, wherein the neurological disease or disorder of the CNS is a non-plaque-forming disease or disorder.

9. The method of claim 8, wherein the non-plaque-forming disease or disorder is selected from the group consisting of Huntington's chorea, viral infections of the brain, brain tumors, lysosomal storage diseases which cause

neurodegeneration and are manifested by enzyme deficiencies, and multiple sclerosis.

10. The method of claim 8, wherein the non-plaque-forming disease or disorder associated with the formation of Lewy bodies.

11. The method of claim 8, wherein the therapeutic molecule displayed on the viral display vehicle is a polypeptide comprising an immunological portion of an antibody that binds at least one epitope of a protein associated with the neurological disease or disorder to inhibit the activity or effect of the protein.

12. The method of claim 8, wherein the therapeutic molecule is displayed on the viral display vehicle via binding or chemical linkage to the surface of the viral display vehicle.

13. The method of claim 1, wherein the viral display vehicle is a filamentous bacteriophage.

14. The method of claim 13, wherein the filamentous bacteriophage is selected from the group consisting of fd, f88, f1, and M13.

15. A pharmaceutical composition for treating a neurological disease or disorder of the central nervous system (CNS), comprising a pharmaceutically acceptable carrier and an effective amount of a viral display vehicle displaying a

therapeutic molecule and being capable of treating a neurological disease or disorder of the CNS.

16. The pharmaceutical composition of claim 15, wherein the viral display vehicle is a filamentous bacteriophage.

17. A method of diagnosing the presence or extent of a neurological disease or disorder of the central nervous system (CNS) by *in vivo* imaging, comprising:

displaying on a viral display vehicle a diagnostic agent capable of being detected by *in vivo* imaging;

introducing the viral display vehicle into a subject by applying the viral display vehicle displaying the diagnostic agent to an olfactory system of the subject; and

detecting the displayed diagnostic agent in the subject by *in vivo* imaging to diagnose the presence or extent of the neurological disease or disorder.

18. The method of claim 17, wherein the viral display vehicle further displays a targeting agent.

19. The method of claim 18, wherein the targeting agent is a ligand of a molecular epitope in the brain useful for diagnosis.

20. The method of claim 17, wherein the diagnostic agent is a radioisotope.

21. The method of claim 17, wherein the diagnostic agent is a contrast agent.

22. The method of claim 17, wherein the *in vivo* imaging is magnetic resonance imaging (MRI).

23. The method of claim 17, wherein the neurological disease or disorder is a plaque-forming disease or disorder.

24. The method of claim 23, wherein the plaque-forming disease or disorder is Alzheimer's disease.

25. The method of claim 24, wherein the targeting agent is selected from the group consisting of Chrysamine-G and a polypeptide comprising an immunological portion of an antibody that binds at least one epitope of beta-amyloid.

26. The method of claim 23, wherein the plaque-forming disease or disorder is associated with the presence of a scrapie isoform (PrP^{Sc}) of prion protein in plaques.

27. The method of claim 26, wherein the targeting agent is a polypeptide comprising an immunological portion of an antibody that binds at least one epitope of prior protein.

28. The method of claim 17, wherein the viral display vehicle is a filamentous bacteriophage.

29. The method of claim 28, wherein the filamentous bacteriophage is selected from the group consisting of fd, f88, f1, and M13.

30. A pharmaceutical composition for diagnosing the presence or extent of a neurological disease or disorder of the central nervous system, comprising a pharmaceutically acceptable carrier and an effective amount of a viral display vehicle which displays a diagnostic agent capable of being detected by *in vivo* imaging.

31. The pharmaceutical composition of claim 30, wherein the viral display vehicle further displays a targeting agent.

32. The pharmaceutical composition of claim 30, wherein the viral display vehicle is a filamentous bacteriophage.

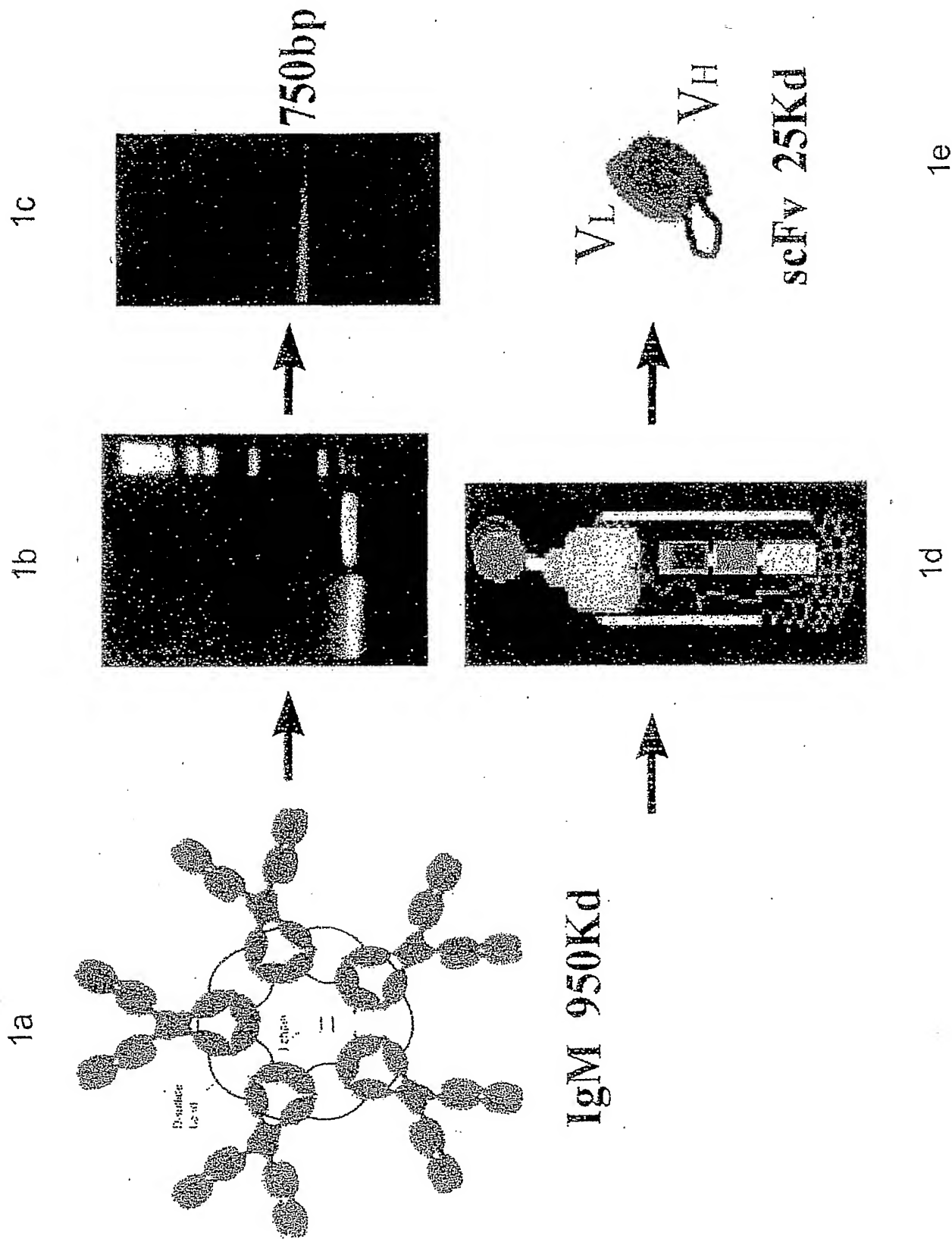


FIGURE 1

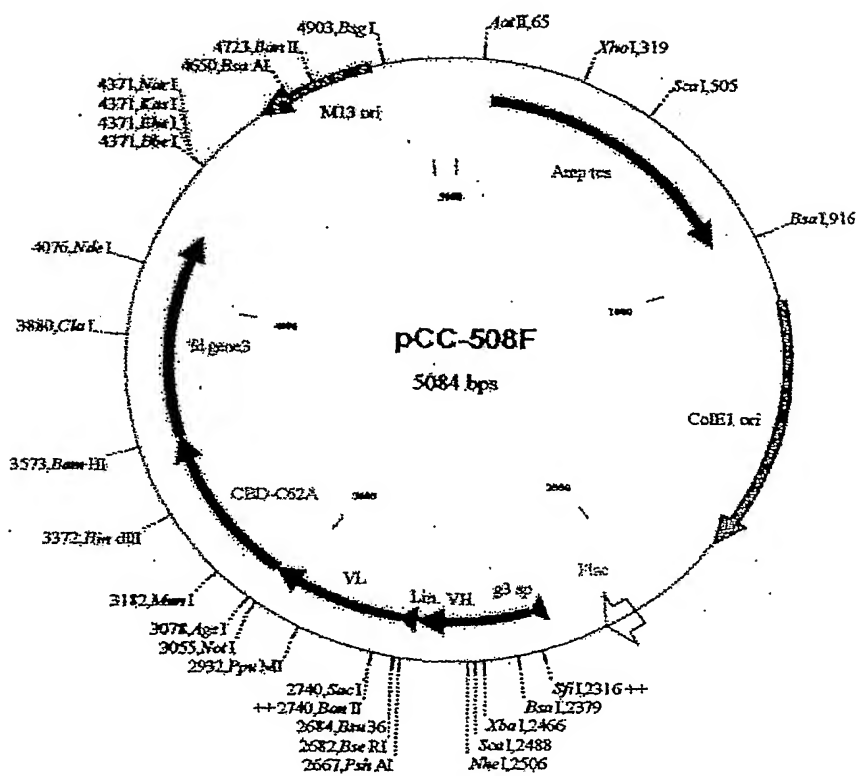


FIGURE 2

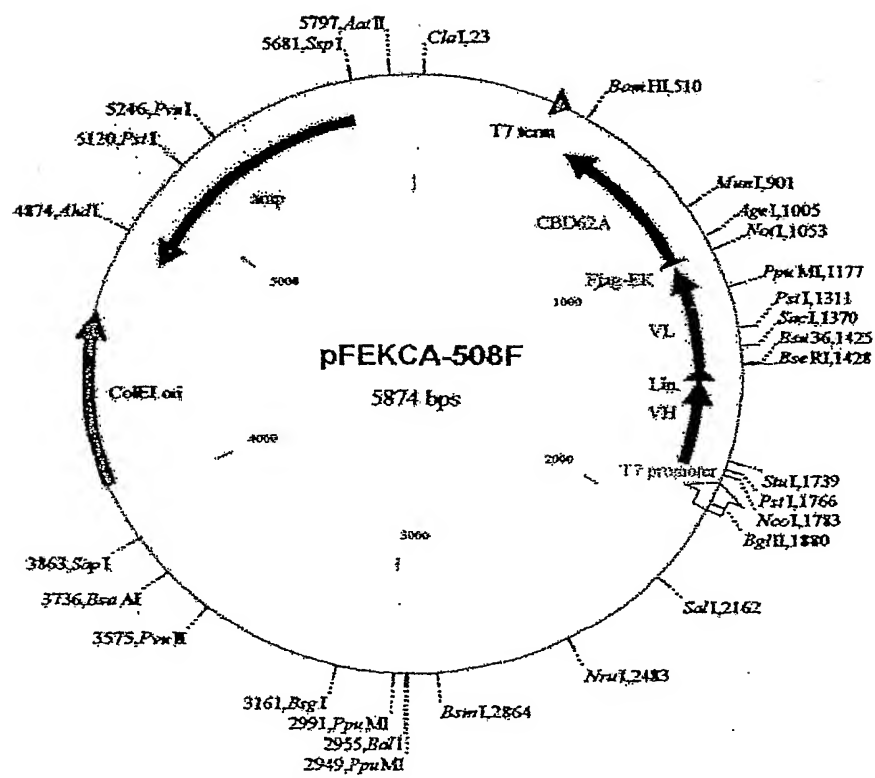


FIGURE 3

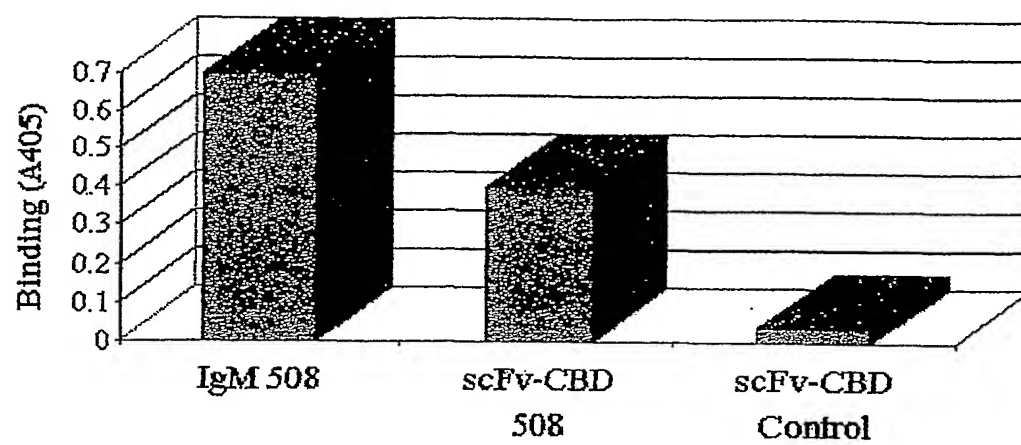


FIGURE 4

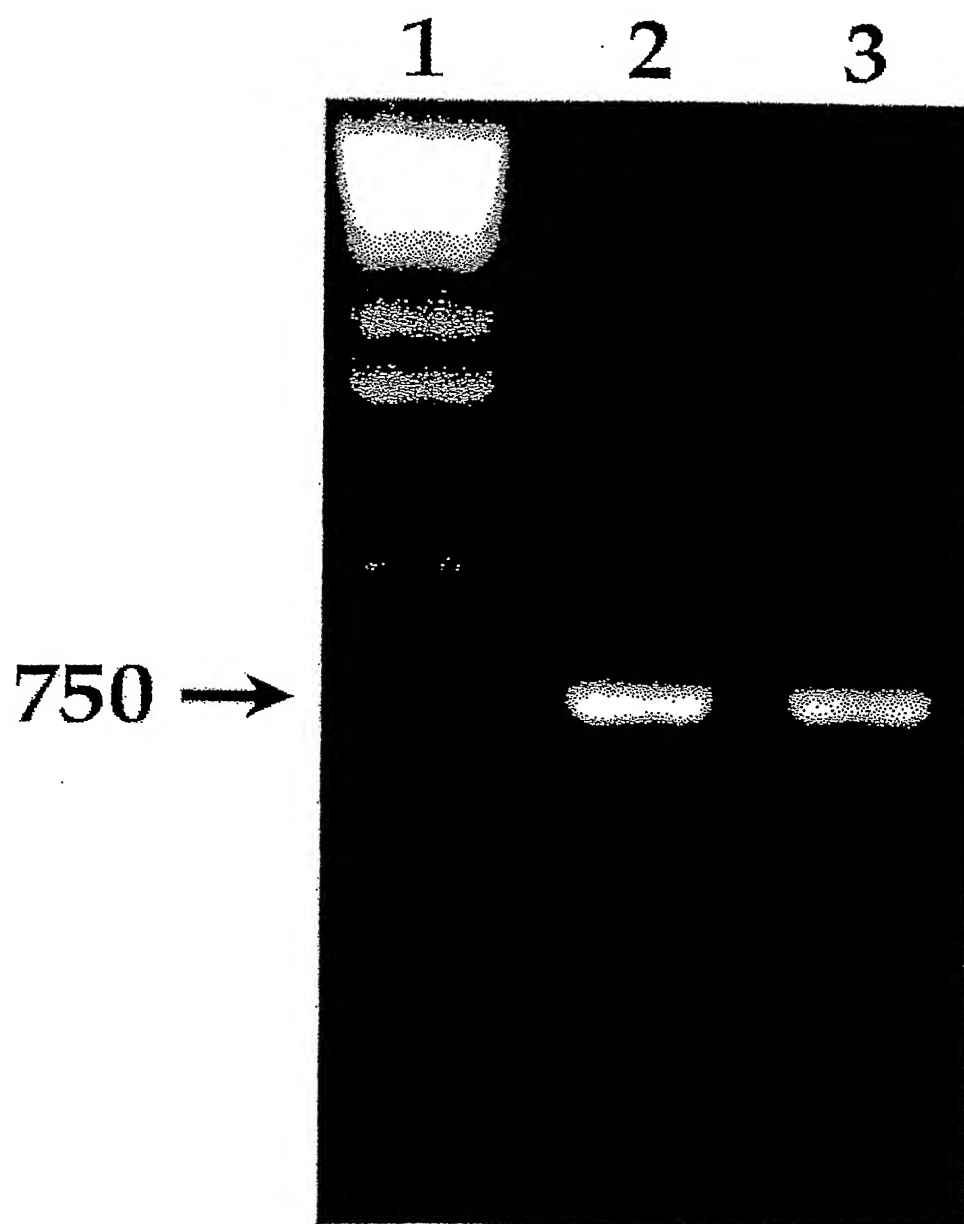


FIGURE 5

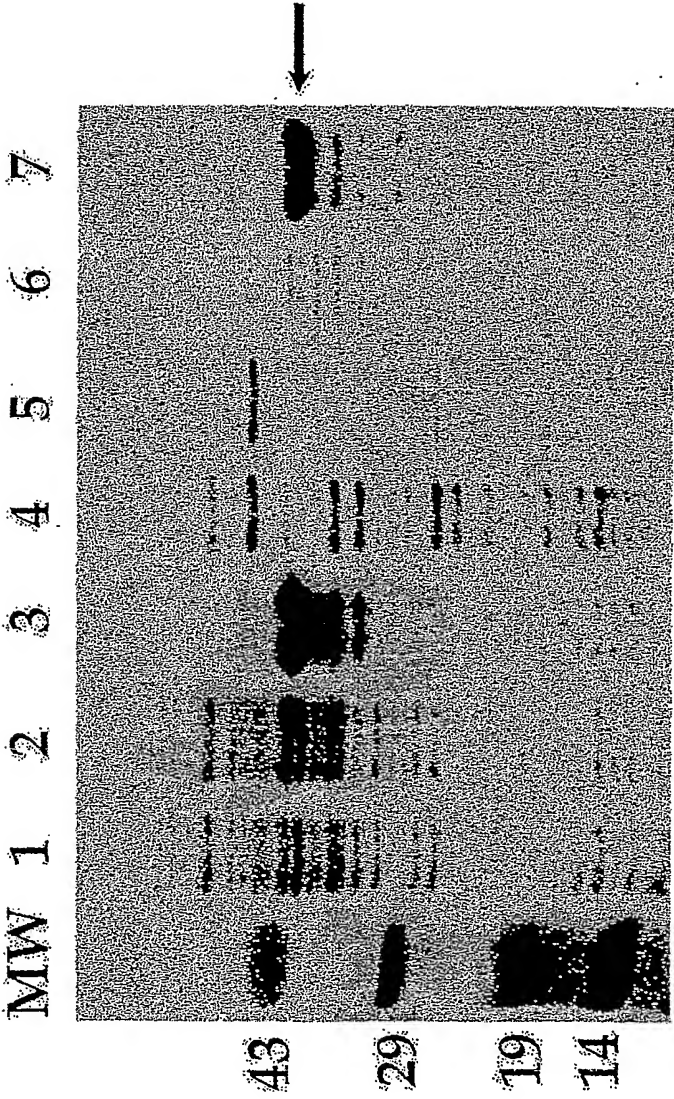
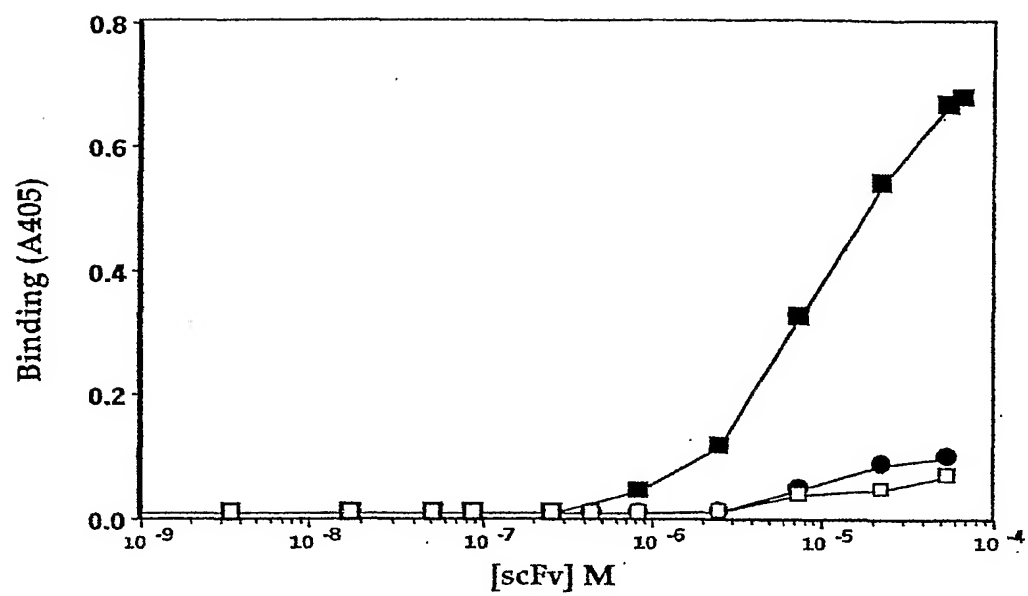


FIGURE 6

**FIGURE 7**

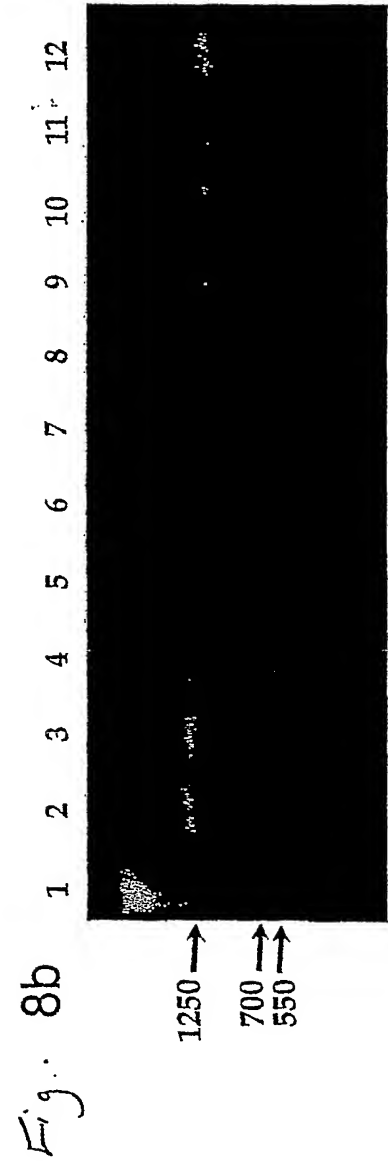
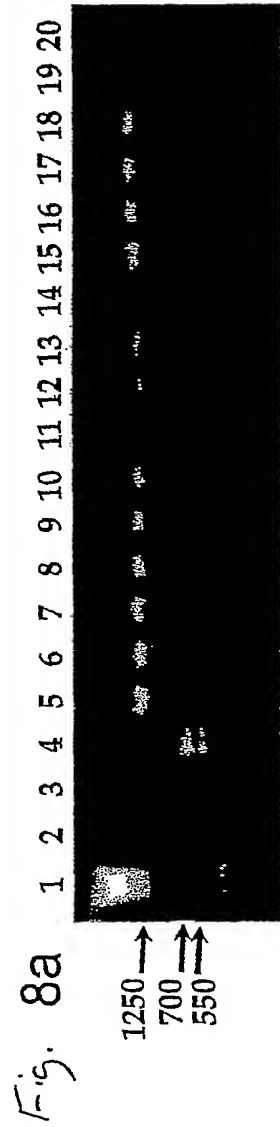


Fig. 9a

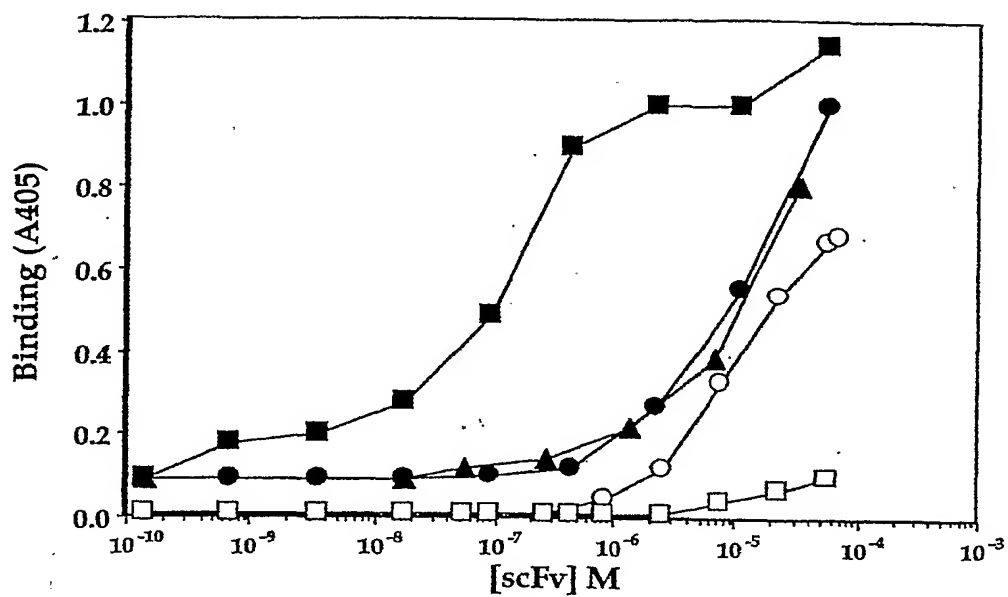
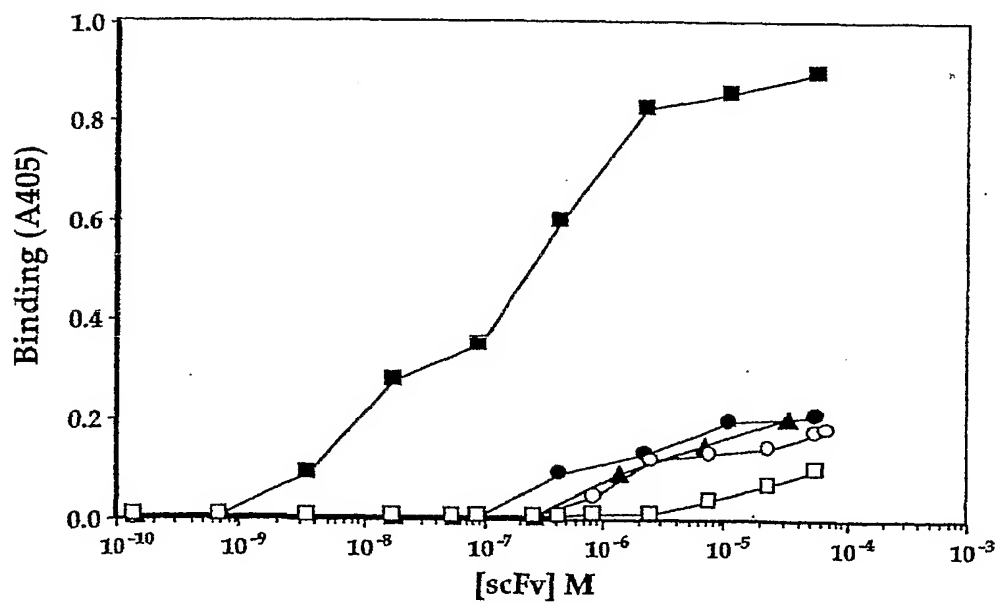
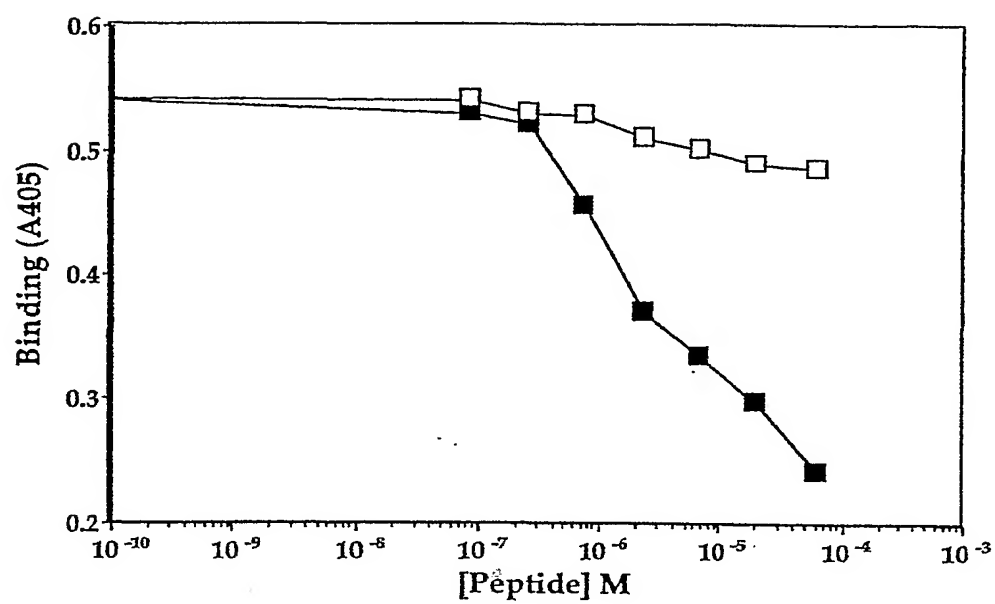


Fig. 9b



**FIGURE 10**

11/30

Fig. 11a.

CAG GTC AAA CTG CAG GAG TCA GGG GCT CAG CTG GTG AGG CCT GGG GTC TCA GTG AAG ATT
 gln val lys leu gln glu ser gly ala glu leu val arg pro gly val ser val lys ile
 TCC TGC AAG GGT TCT GGC TAC ACA TTC ACT GAT TAT GCT ATG CAC TGG GTG AAG CAG AGT
 ser cys lys gly ser gly tyr thr phe thr asp tyr ala met his trp val lys gln ser
CDR 1
 CAT GCA AAG AGT CTA CAG TGG ATT GCA GTT ATT AGT ACT TAC TAT GGT GAT GCT AGC TAC
 his ala lys ser leu glu trp ile gly val ile ser thr tyr tyr gly asp ala ser tyr
CDR 2
 AAC CAG AAG TTC AAG GGC AAG GCC ACA ATG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAT
asn gln lys phe lys gly lys ala thr met thr val asp lys ser ser ser thr ala tyr
CDR 2
 ATG GAA CTT GCC AGA CTG ACA TCT GAG CAT TCT GCC ATC TAT TAC TGT GCA AGA GGG GCT
 met glu leu ala arg leu thr ser glu asp ser ala ile tyr tyr cys ala arg gly ala
CDR 3
 ACT ATG TCC TAC TTT GAC TAC TGG GGC CAA GTG ACC ACG GTC AOC GTC TCC TCA ggt gga
 thr met ser tyr phe asp tyr trp gly gln val thr thr val thr val ser ser gly gly
CDR 3

Fig. 11b.

ggc ggt tca ggc gga gtt ggc tct ggc ggt ggc gga tgg GAC ATC GAG CTC ACT CAG TCT
 gly gly ser gly gly val gly ser gly gly gly gly ser asp ile glu leu thr gln ser
Linker
 CCA GCA ATC ATG TCT GCA TTT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT GCC AGC TCA
 pro ala ile met ser ala ser pro gly glu lys val thr met thr cys ser ala ser ser
CDR 1
 AGT ATA AGT TAC ATG CAC TGG TAT CAG CAG AAG CCA GGC ACC TCC CCC AAA AGA TGG ATT
 ser ile ser tyr met his trp tyr gln gln lys pro gly thr ser pro lys arg trp ile
CDR 1
 TAT GAC ACA TCC AAA CTG GCT TCT GGA GTC CCT GCT GGC TTC AGT GGC AGT GGG TCT GGG
 tyr asp thr ser lys leu ala ser gly val pro ala arg phe ser gly ser gly ser gly
CDR 2
 ACC TCT TAT TCT CTC ACA ATC AGC ACC ATG GAG GCT GAA CAT GCT GCC ACT TAT TAC TGC
 thr ser tyr ser leu thr ile ser ser met glu ala glu asp ala ala thr tyr tyr cys
 CAT CAG CCG AGT AGT TAC CCA TTC ACG TTC GGA GGG GGC GCC AAG CTG GAA ATA AAA
 his gln arg ser ser tyr pro phe thr phe gly gly gly ala lys leu glu ile lys
CDR 3

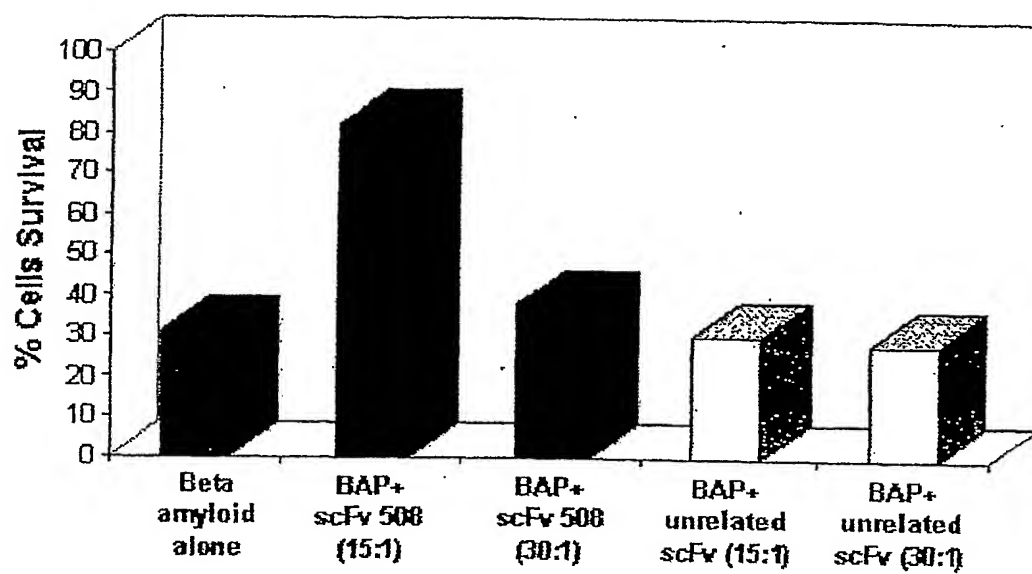


FIGURE 12

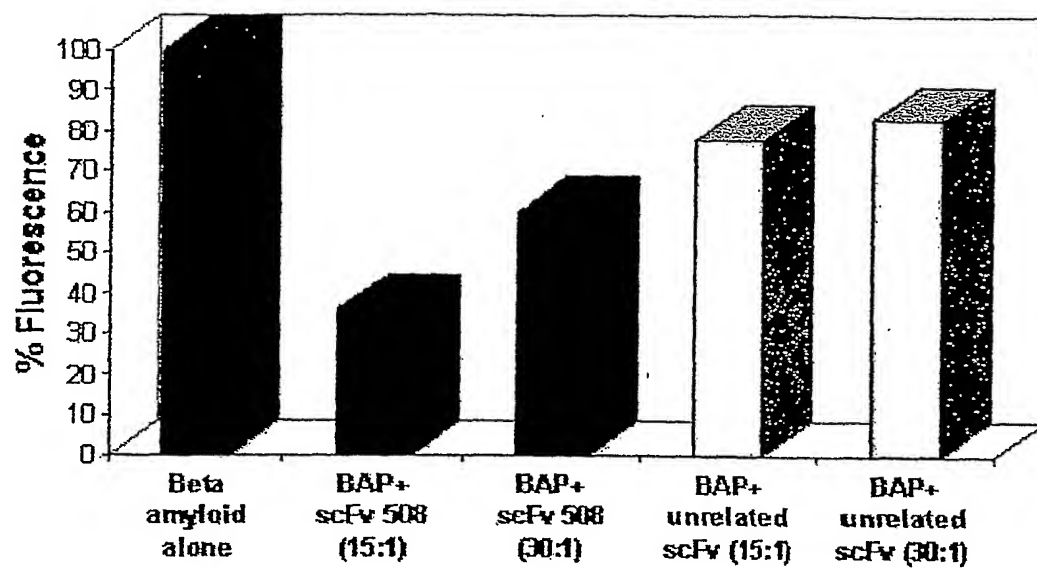


FIGURE 13

Fig. 14c

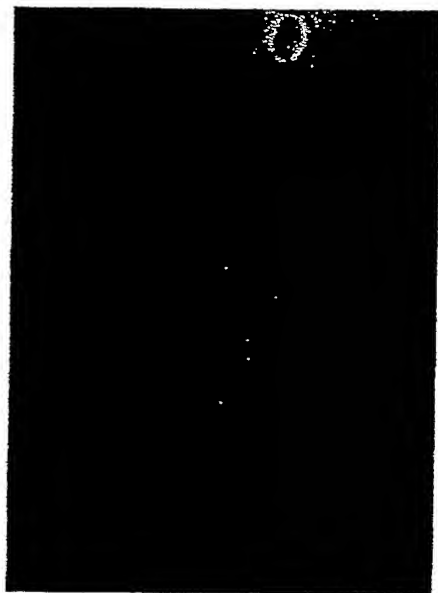


Fig. 14d

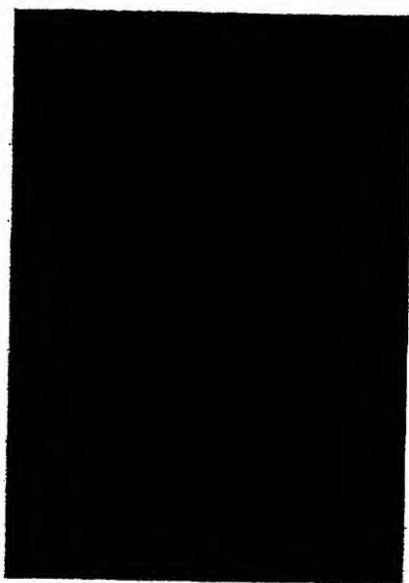


Fig. 14a

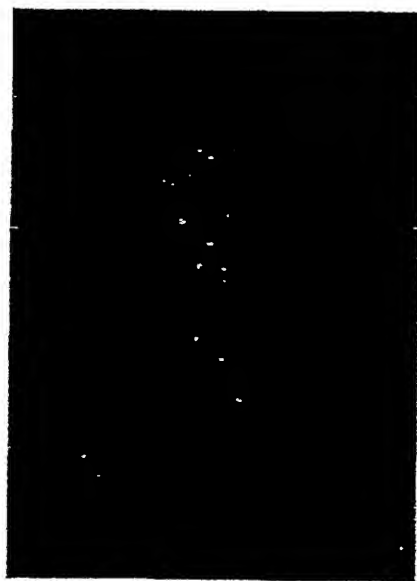


Fig. 14b



Fig 15c

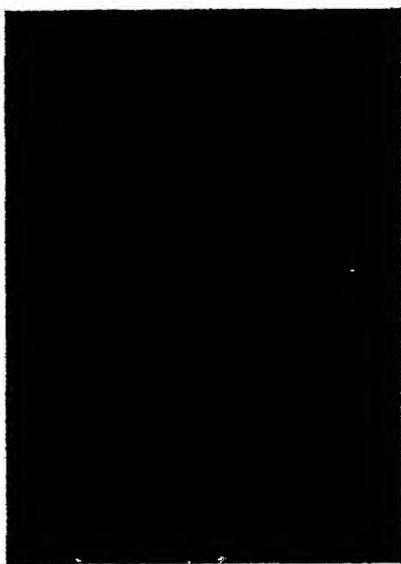


Fig 15d

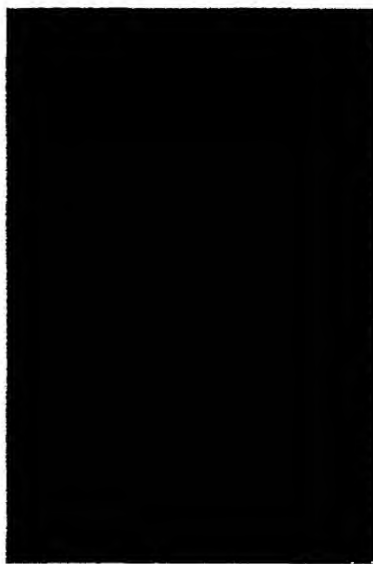


Fig 15a

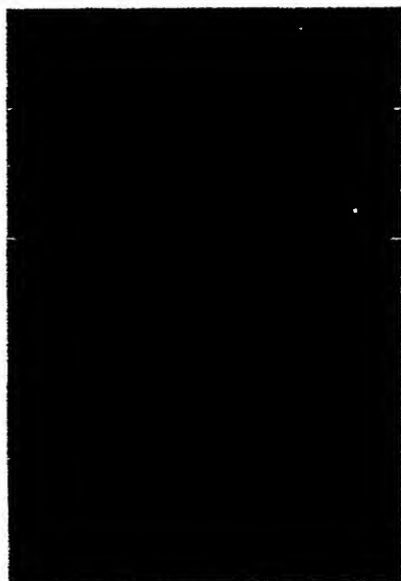


Fig 15b



Fig. 16c

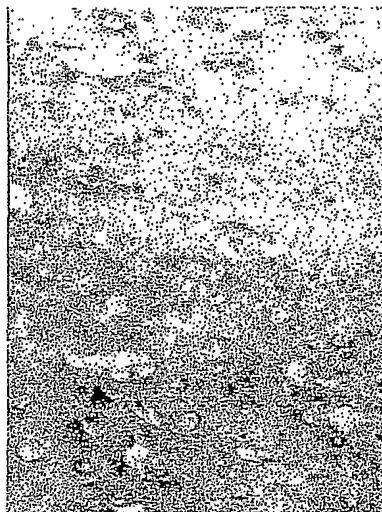


Fig. 16d

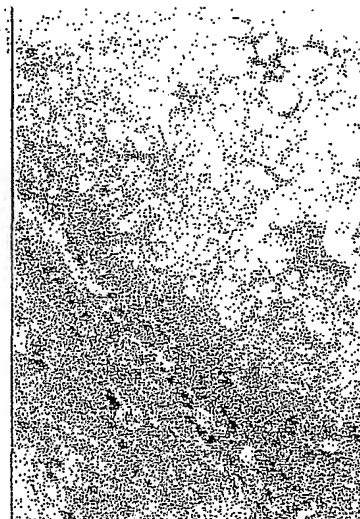


Fig. 16a



Fig. 16b

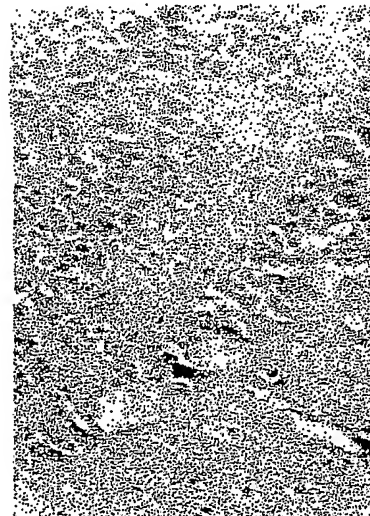


Fig. 17c

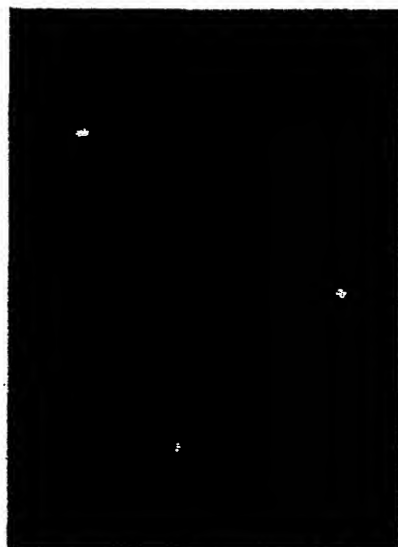


Fig. 17d

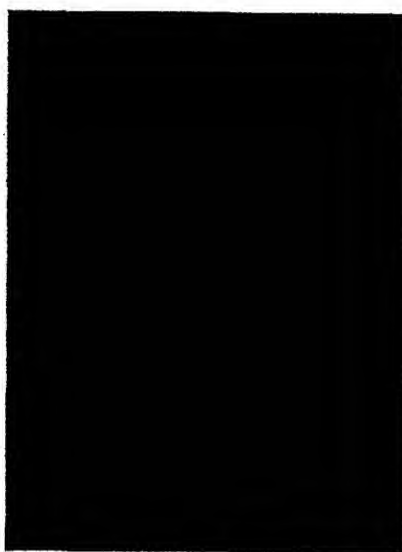
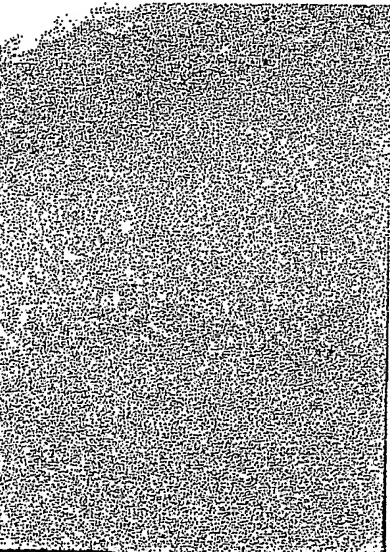
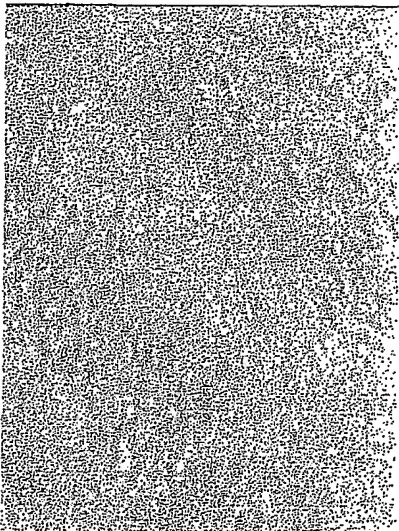
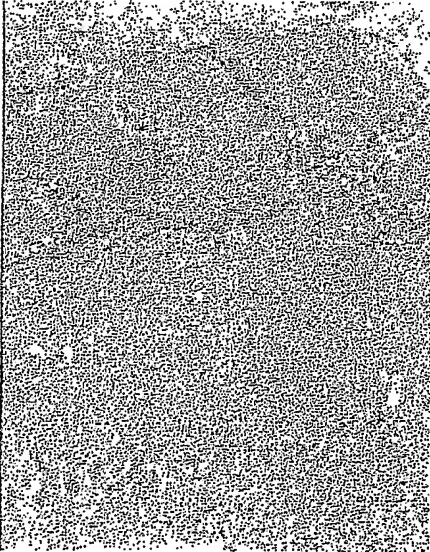
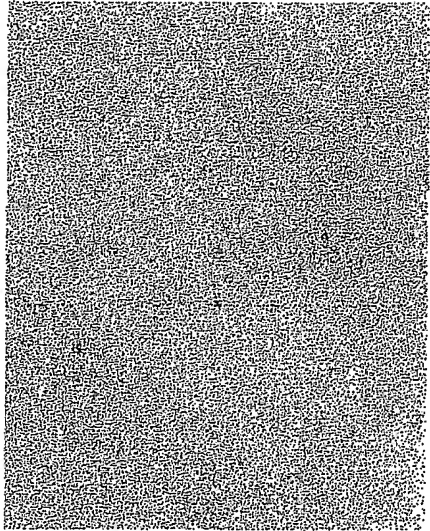


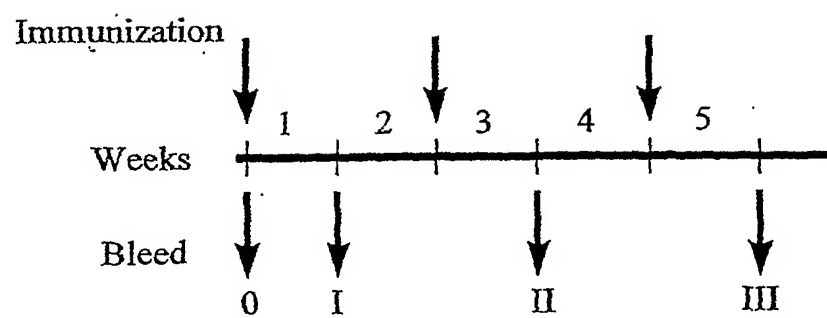
Fig. 17a



Fig. 17b





**FIGURE 19**

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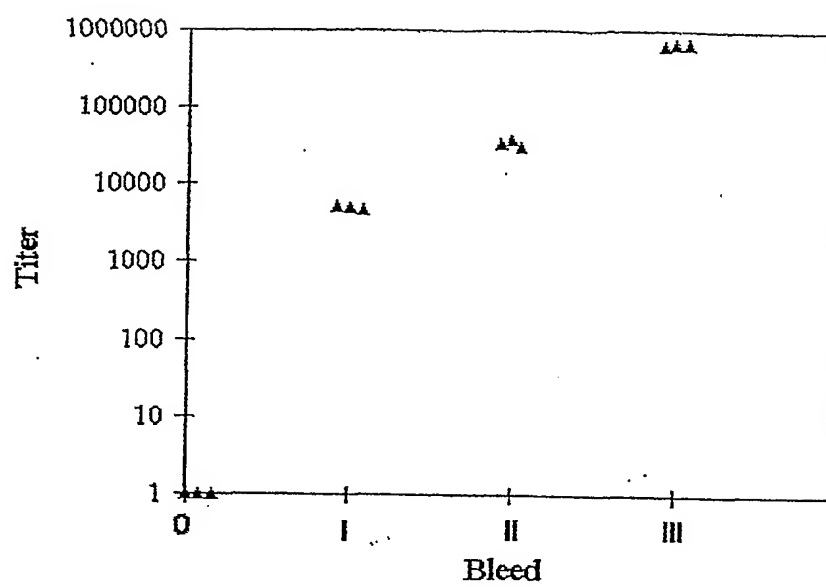
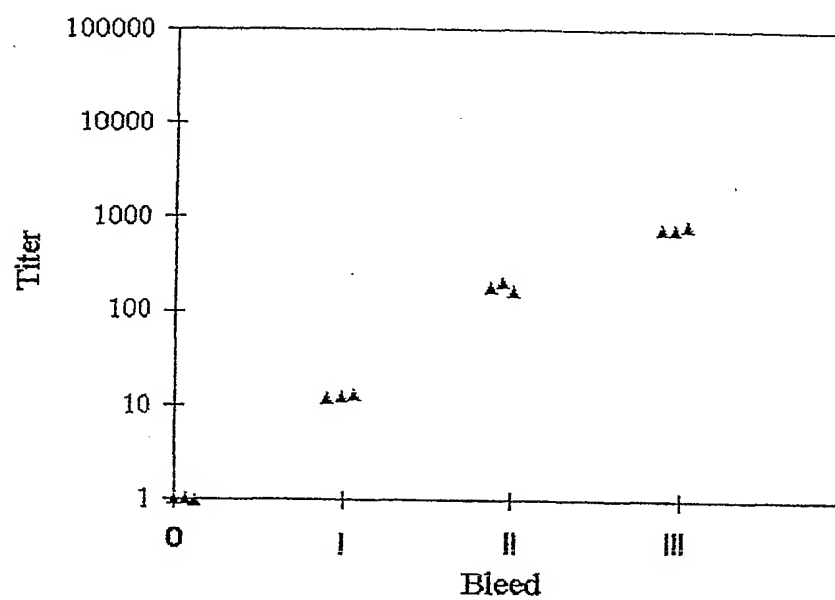


Fig. 20b



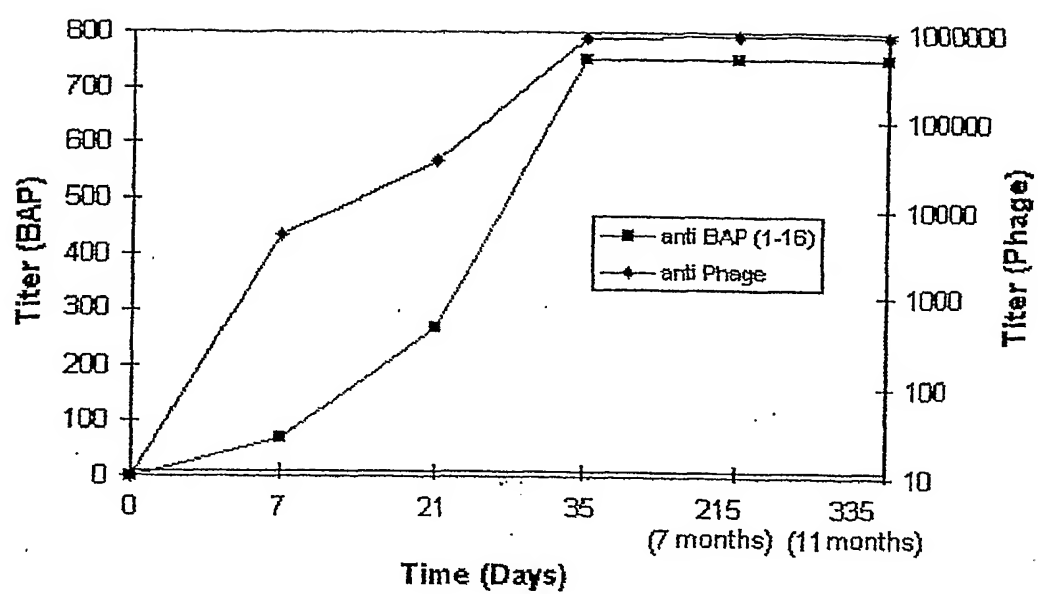


FIGURE 21

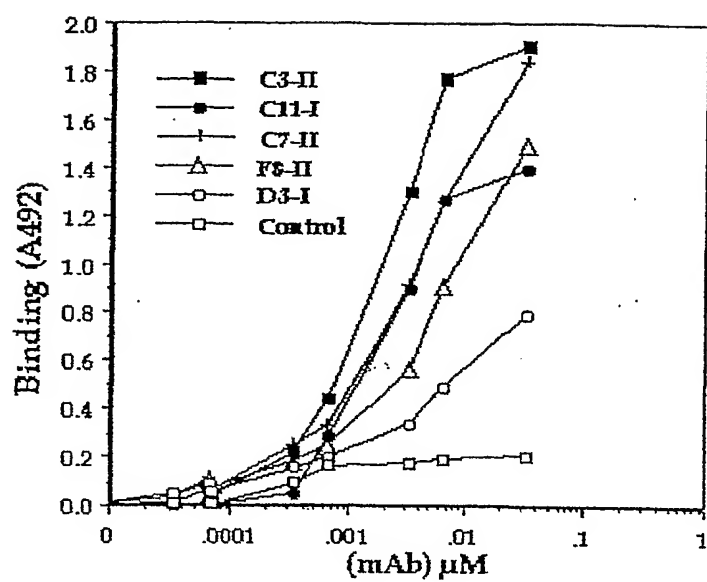


FIGURE 22

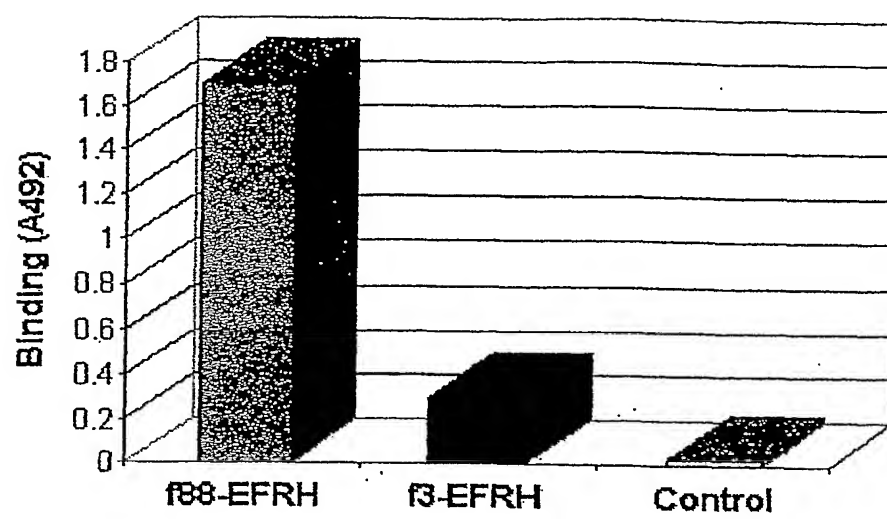


FIGURE 23

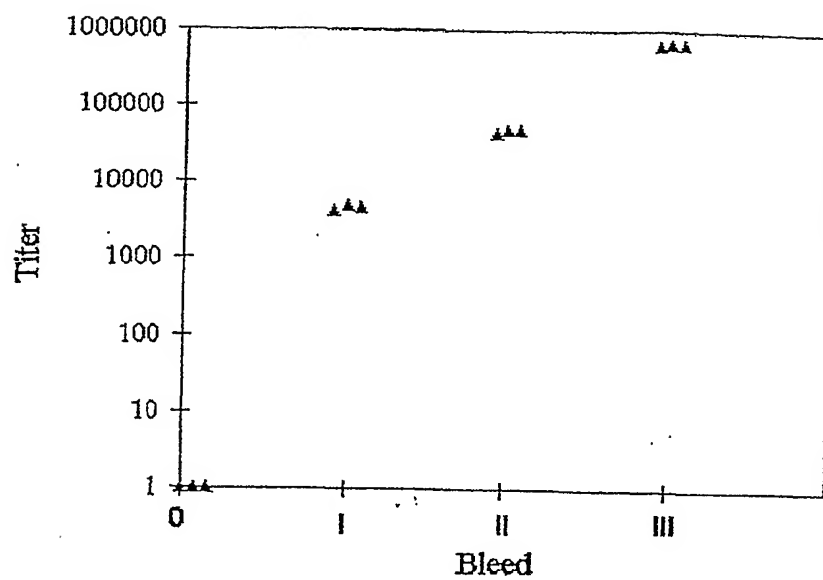


Fig 24a

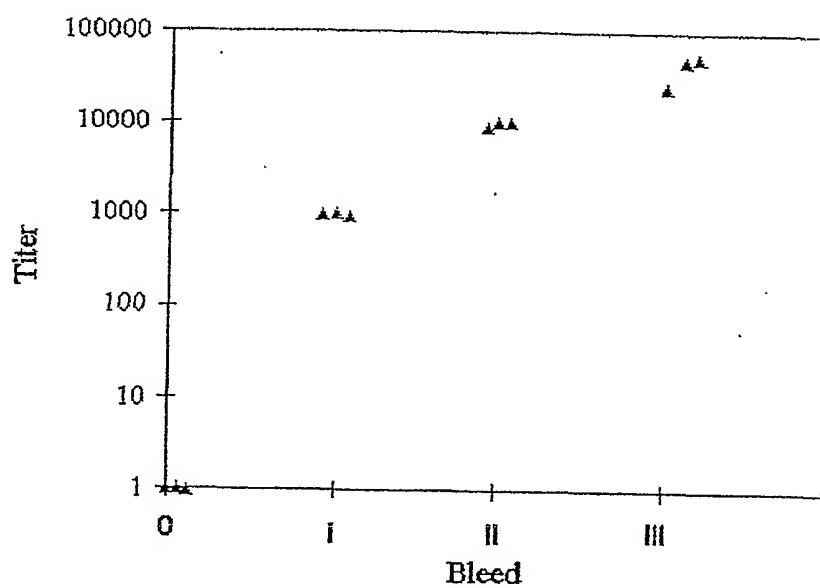


Fig 24b

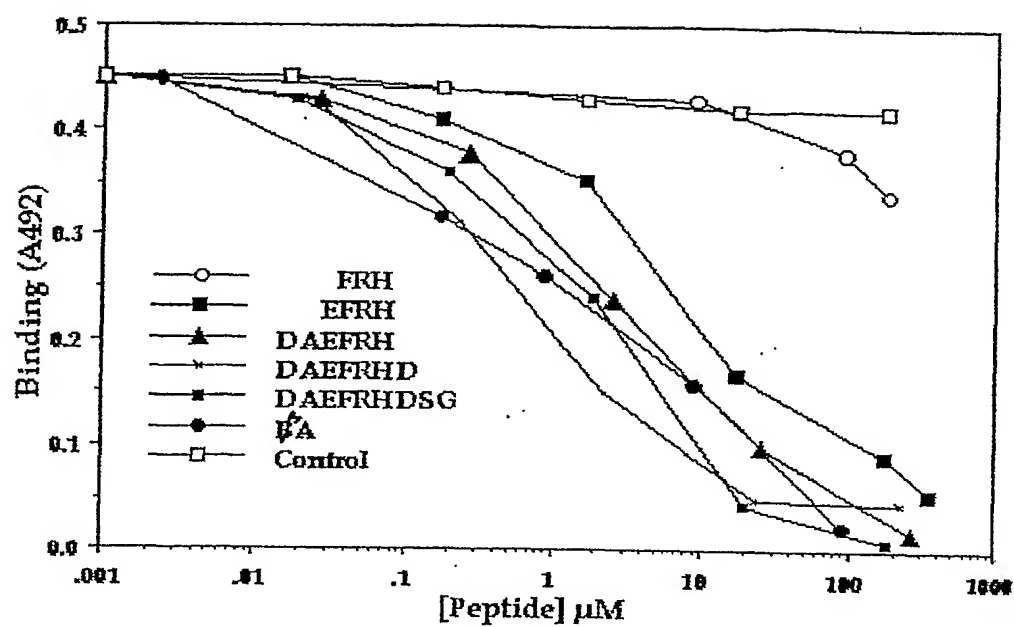


FIGURE 25

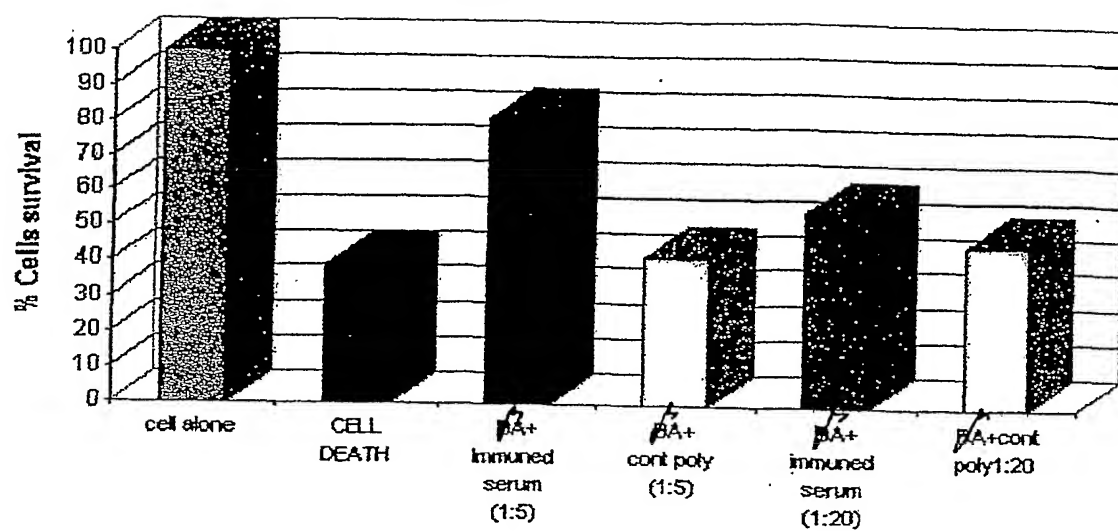


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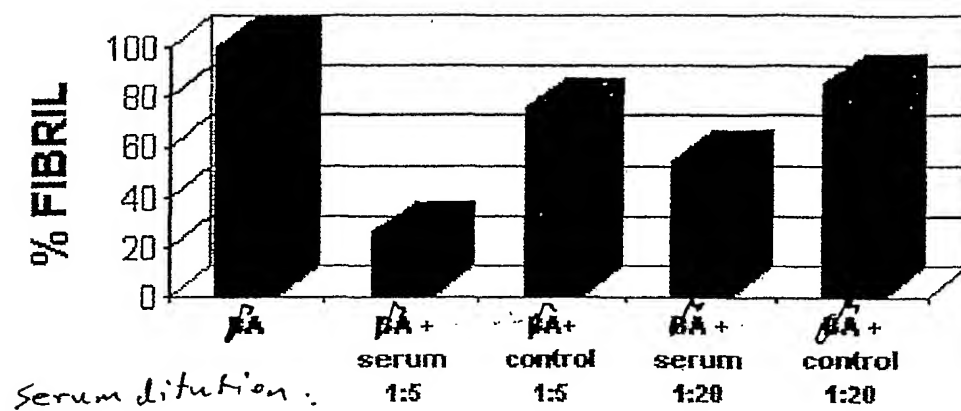


FIGURE 27

| | | |
|-------|--------------|---|
| Human | PrP 106-126: | KTN M KH M AGAAAAGAVVGGLG |
| Mouse | PrP 105-125: | KTN L KH V AGAAAAGAVVGGLG |

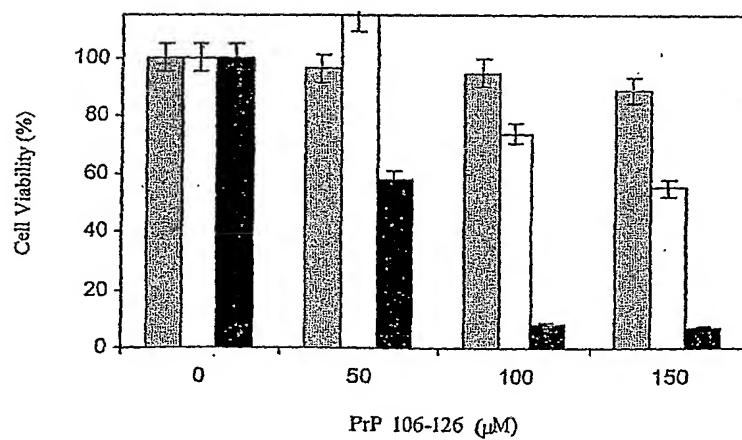
Fig 28Fig 29

Figure 30

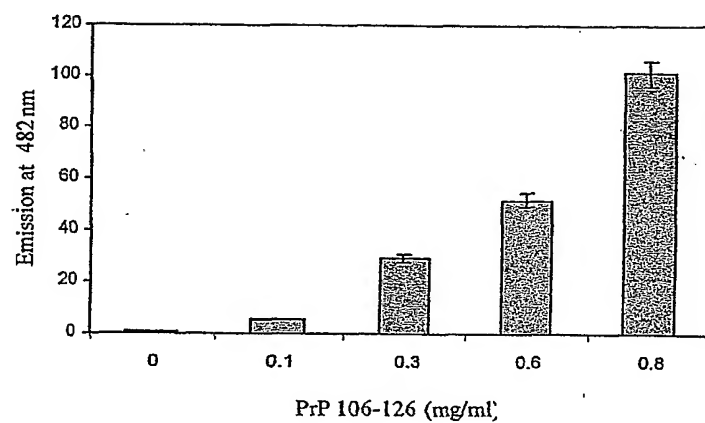
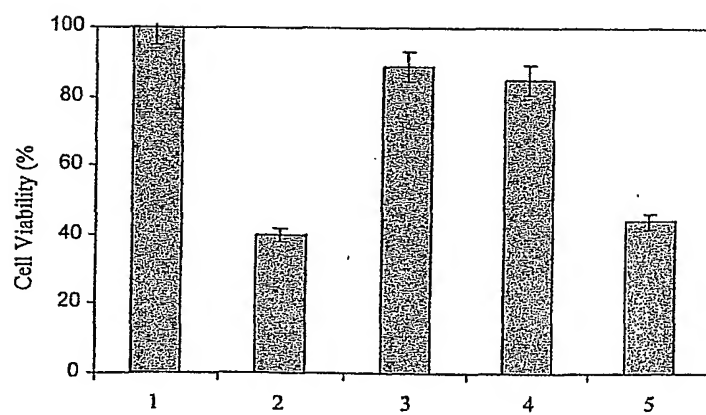
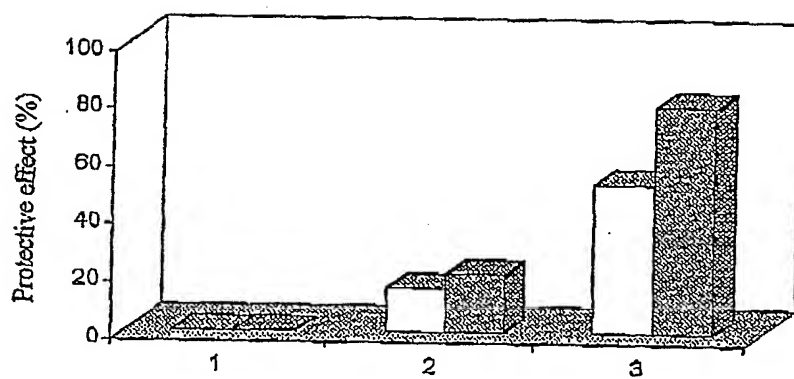
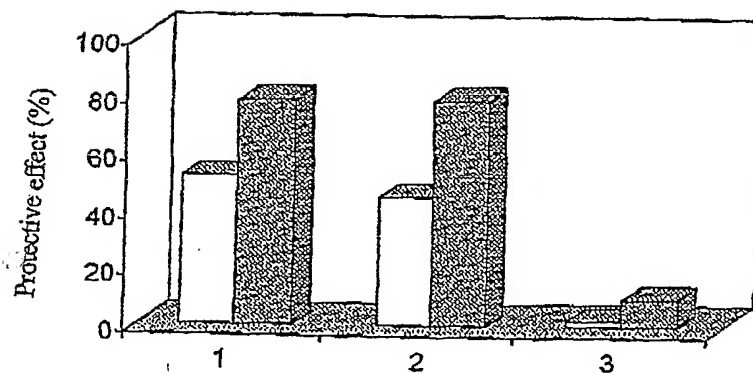


Figure 31



Fig 32Fig 33

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HANAN, Eilat

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<150> 09/629,971

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<150> US 09/473,653

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10

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1

5

10

15

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 tca gtg aag att tcc tgc aag ggt tct ggc tac aca ttc act gat tat 96
 Ser Val Lys Ile Ser Cys Lys Gly Ser Gly Tyr Thr Phe Thr Asp Tyr
 20 25 30
 gct atg cac tgg gtg aag cag agt cat gca aag agt cta gag tgg att 144
 Ala Met His Trp Val Lys Gln Ser His Ala Lys Ser Leu Glu Trp Ile
 35 40 45
 gga gtt att agt act tac tat ggt gat gct agc tac aac cag aag ttc 192
 Gly Val Ile Ser Thr Tyr Tyr Gly Asp Ala Ser Tyr Asn Gln Lys Phe
 50 55 60
 aag ggc aag gcc aca atg act gta gac aaa tcc tcc agc aca gcc tat 240
 Lys Gly Lys Ala Thr Met Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80
 atg gaa ctt gcc aga ctg aca tct gag gat tct gcc atc tat tac tgt 288
 Met Glu Leu Ala Arg Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys
 85 90 95
 gca aga ggg gct act atg tcc tac ttt gac tac tgg ggc caa gtg acc 336
 Ala Arg Gly Ala Thr Met Ser Tyr Phe Asp Tyr Trp Gly Gln Val Thr
 100 105 110
 acg gtc acc gtc tcc tca ggt gga ggc ggt tca ggc gga gtt ggc tct 384
 Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Val Gly Ser
 115 120 125
 ggc ggt ggc gga tcg gac atc gag ctc act cag tct cca gca atc atg 432
 Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met

| 130 | 135 | 140 | |
|---|-----|-----|-----|
| tct gca tct cca ggg gag aag gtc acc atg acc tgc agt gcc agc tca | | | 480 |
| Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser | | | |
| 145 | 150 | 155 | 160 |
| agt ata agt tac atg cac tgg tat cag cag aag cca ggc acc tcc ccc | | | 528 |
| Ser Ile Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Thr Ser Pro | | | |
| | 165 | 170 | 175 |
| aaa aga tgg att tat gac aca tcc aaa ctg gct tct gga gtc cct gct | | | 576 |
| Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala | | | |
| | 180 | 185 | 190 |
| cgc ttc agt ggc agt ggg tct ggg acc tct tat tct ctg aca atc agc | | | 624 |
| Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser | | | |
| | 195 | 200 | 205 |
| agc atg gag gct gaa gat gct gcc act tat tac tgc cat cag cgg agt | | | 672 |
| Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Arg Ser | | | |
| | 210 | 215 | 220 |
| agt tac cca ttc acg ttc gga ggg ggg gcc aag ctg gaa ata aaa | | | 717 |
| Ser Tyr Pro Phe Thr Phe Gly Gly Gly Ala Lys Leu Glu Ile Lys | | | |
| 225 | 230 | 235 | |

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| 1 | 15 |
| Ser Val Lys Ile Ser Cys Lys Gly Ser Gly Tyr Thr Phe Thr Asp Tyr | |
| 20 | 30 |
| Ala Met His Trp Val Lys Gln Ser His Ala Lys Ser Leu Glu Trp Ile | |
| 35 | 45 |
| Gly Val Ile Ser Thr Tyr Tyr Gly Asp Ala Ser Tyr Asn Gln Lys Phe | |
| 50 | 60 |
| Lys Gly Lys Ala Thr Met Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr | |
| 65 | 80 |
| Met Glu Leu Ala Arg Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys | |
| 85 | 95 |
| Ala Arg Gly Ala Thr Met Ser Tyr Phe Asp Tyr Trp Gly Gln Val Thr | |
| 100 | 110 |
| Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Val Gly Ser | |
| 115 | 125 |

Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met
 130 135 140

Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser
 145 150 155 160

Ser Ile Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Thr Ser Pro.
 165 170 175

Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala
 180 185 190

Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser
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Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Arg Ser
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Ser Tyr Pro Phe Thr Phe Gly Gly Gly Ala Lys Leu Glu Ile Lys
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atctatgcgg cccagccggc catg 24

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His Gln Arg Ser Ser Tyr Pro Cys Thr
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<400> 24

| | | | | | | | | | | | | | | |
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| 1 | | | | 5 | | | | | 10 | | | | | 15 |

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| Lys | Thr | Asn | Met | Lys | His | Met | Ala | Gly | Ala | Ala | Ala | Ala | Gly | Ala | Val |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |

| | | | | |
|-----|-----|-----|-----|-----|
| Val | Gly | Gly | Leu | Gly |
| | | | 20 | |

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| ggc | ggt | tca | ggc | gga | ggt | ggc | tct | ggc | ggt | ggc | gga | tcg | gac | atc | gag | 48 |
| Gly | Gly | Ser | Gly | Gly | Val | Gly | Ser | Gly | Gly | Gly | Gly | Ser | Asp | Ile | Glu | |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | | |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| ctc | act | cag | tct | cca | gca | atc | atg | tct | gca | tct | cca | ggg | gag | aag | gtc | 96 |
| Leu | Thr | Gln | Ser | Pro | Ala | Ile | Met | Ser | Ala | Ser | Pro | Gly | Glu | Lys | Val | |
| | | | 20 | | | | | 25 | | | | | 30 | | | |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| acc | atg | acc | tgc | agt | gcc | agc | tca | agt | ata | agt | tac | atg | cac | tgg | tat | 144 |
| Thr | Met | Thr | Cys | Ser | Ala | Ser | Ser | Ser | Ile | Ser | Tyr | Met | His | Trp | Tyr | |
| | | 35 | | | | | 40 | | | | | 45 | | | | |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| cag | cag | aag | cca | ggc | acc | tcc | ccc | aaa | aga | tgg | att | tat | gac | aca | tcc | 192 |
| Gln | Gln | Lys | Pro | Gly | Thr | Ser | Pro | Lys | Arg | Trp | Ile | Tyr | Asp | Thr | Ser | |
| | | 50 | | | | 55 | | | | | 60 | | | | | |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| aaa | ctg | gct | tct | gga | gtc | cct | gct | cgc | ttc | agt | ggc | agt | ggg | tct | ggg | 240 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
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 acc tct tat tct ctc aca atc agc agc atg gag gct gaa gat gct gcc 288
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 act tat tac tgc cat cag cgg agt agt tac cca ttc acg ttc gga ggg 336
 Thr Tyr Tyr Cys His Gln Arg Ser Ser Tyr Pro Phe Thr Phe Gly Gly
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 35 40 45
 Gln Gln Lys Pro Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser
 50 55 60
 Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
 65 70 75 80
 Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala
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 Gly Ala Lys Leu Glu Ile Lys
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Val Gly Gly Leu Gly
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His His His His His His
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